MARIA HELENE PALESA JAEGER

Phenotypic, Quantitative Genetic and Genomic Characterization of the German Black and White Dual-Purpose Cattle Breed

INAUGURAL-DISSERTATION

zur Erlangung des Doktorgrades eines Doctor agriculturae (Dr. agr.)
durch den Fachbereich Agrarwissenschaften, Ökotrophologie und
Umweltmanagement der Justus-Liebig-Universität Gießen

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vorgelegt von

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The key is a man's power of accumulative selection: nature gives successive variations; man adds them up in certain directions useful to him.

Charles Darwin

Meiner Familie

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List of Abbreviations

ABL	Alte Bundesländer
ACT	Daily activity behaviour (sensor trait)
AGG	Aggressiveness
AI	Artificial insemination
AMGG	Annual monetary genetic gain
ASD	Allele sharing distance
AvgBW	Average body weight
BCS	Body condition score
BLUP	Best linear unbiased prediction
BP	Breeding program
BTA	*Bos taurus* (chromosome)
C_BP	Conventional breeding program
CH_OBS	Original Brown Swiss from Switzerland
CH_Si	Simmental from Switzerland
CLA	Conjugated linoleic acid
CM	Clinical mastitis
CN	Casein
d	Day
DC	Discounted costs
DE_DN	Red and White dual-purpose cattle from Germany
DE_DSN	Black and White dual-purpose cattle from Germany
DIM	Days in milk
DMI	Dry matter intake
DO	Days open
DR	Discounted return
DRP	De-regressed proof
DSN	Deutsches Schwarzbuntes Niederungsrind (Black and White dual-purpose cattle)
DSN_90 %	Cows with ≥ 90 % of DSN breed percentage (based on own algorithm)
EBV	Estimated breeding value
ET	Ear temperature
F	Inzuchtkoeffizient, Inbreeding coefficient
F-ALTITUDE	Farm altitude
FDR	False discovery rate
FEED	Daily feeding behaviour (sensor trait)
Fkg	Fat kilogram
F-LATITUDE	Farm latitude
Fat %	Fat percent

FPR	Fat-to-protein ratio
FSG_BP	Combined BP, changing percent. of AI, NSS in female selection group
GT	General temperament
GUI	Genotyp-Umwelt-Interaktion
GWAS	Genome wide association study
GxE	Genotype by environment (interaction)
GxE_BP	Conventional breeding program with GxE interaction
h^2	Heritability
HA	Daily high active behaviour (sensor trait)
H-CA	Average herd calving age
H-DSN %	Average herd DSN percentage
HF	Holstein Friesian cattle
HF_90 %	Cows with ≥ 90 % of HF breed percentage (based on own algorithm)
H-MPL	Average herd milk production level
H-NSS	Average herd natural service sire implementation
H-SCC	Average herd somatic cell count
H-SIZE	Average herd size
I	Index (sensor trait)
IC	Index class (sensor trait)
IHRO	Intra herd rank order
kb	Kilo bases
KB	Künstliche Besamung
L	Longevity
LD	Linkage disequilibrium
LG	Lactoglobulin
LHS	Leg hygiene score
LS	Locomotion score
MACE	Multiple-trait across country evaluation
MAF	Minor allele frequency
MFSG_BP	Combined breeding program with changing percentage of AI and NSS in both male and female selection groups
Mkg	Milk kilogram
mL	Millilitre
MSG_BP	Combined BP, changing percent. of AI and NSS in male selection group
MTAM	Multiple-trait animal model
MY	Milk yield
NA	Daily sleeping (not active) behaviour (sensor trait)
NBL	Neue Bundesländer
NDF	Natural detergent fibre
N_e	Effective population size

NSB	Natursprungbulle
NSS	Natural service sire
O_BP	Organic breeding program
OGC	Optimum genetic contribution (theory)
PCA	Principal component analysis
PDF	Precision dairy farming
PL_BS	Brown Swiss cow from Poland
PL_HF	Holstein Friesian cow from Poland
Pro %	Protein percent
QTL	Quantitative trait loci
R	Average relationship
r^2	Reliability of estimated breeding value
RA	Rasseanteil
RBB	Rinderzuchtverband Berlin-Brandenburg eG
RC	Rasse Code
RFI	Residual feed intake
r_g	Genetic correlation
r_p	Phenotypic correlation
RUM	Daily rumination behaviour (sensor trait)
SCC	Somatic cell count
SCS	Somatic cell score
Sl_BS	Brown Swiss cow from Slovenia
Sl_HF	Holstein Friesian cow from Slovenia
Sl_Si	Simmental from cow Slovenia
SMR	Schwarzbuntes Milchrind
SNP	Single nucleotide polymorphism
THI	Temperature humidity index
UHS	Udder hygiene score
VEF-DSN	Verein zur Erhaltung und Förderung des alten schwarzbunten Niederungsrindes
VIT	Vereinigte Informationssysteme Tierhaltung e.V.
WAT	Welfare assessment traits

SUMMARY

The objective of this study was an in-depth evaluation of novel functional traits in endangered dual-purpose cattle, kept in organic pasture-based production systems, on a quantitative genetic, as well as a genomic level, including the effect of environmental influences. The preliminary work constituted a comprehensive pedigree analysis within the endangered black and white dual-purpose (DSN) cattle breed, along with a detailed examination of the population structure (inbreeding, genetic relationships, effective population size), to better characterize the German DSN population. Against this background, a simulation of breeding programs and economic evaluations of production and functional traits of the DSN breed was conducted, based on deterministic equations and population parameters. Different aspects of DSN breeding are divided into five scientific studies (**chapters 2**, **3**, **4**, **5** and **6**), which address the previously mentioned research areas: 1) Characterization of the German DSN population, based on pedigree analyses; 2) Examination of relations between novel functional traits and productivity, health and welfare indicator traits in native DSN cattle, under grazing conditions; 3) Multi-breed genome wide association studies (GWAS) of novel functional traits in DSN and other European dual-purpose cattle; 4) Variance heterogeneity and genotype by environment (GxE) interaction analysis in DSN for different herd allocation schemes; 5) Comparison of disparate breeding scenarios for the German DSN population. In **chapter 7**, further aspects of quantitative-genetic and genomic studies are presented and discussed against the background of DSN breeding program improvements. Finally, genetic correlations between novel functional and production traits are estimated and an outlook regarding future challenges and trends in German DSN breeding is given.

As the last in-depth genetic population evaluation of the endangered German DSN breed was conducted more than 15 years ago, gaining an up-to-date status quo of this situation was essential, before addressing all subsequent research questions. Thus, in the first study (**chapter 2**), an own algorithm was developed, in order to identify DSN and Holstein Friesian (HF) cows, according to their genetic percentages. These newly calculated breed percentages were the basis for further investigations on German HF and DSN population genetics (relationship within and between defined groups, inbreeding coefficients, effective population size, generation intervals, and possible inbreeding depressions). The

newly developed algorithm revealed pronounced differences between animal allocations to breeds, based on their genetic percentage, compared to allocations, based on the officially assigned breed codes. Considering genetic breed percentages and strict thresholds of 90 % breed percentage, 46 % of cows were wrongly ascribed to DSN (compared to their official breed code). Moreover, there was a distinct difference between wrongly allocated DSN in eastern and western Germany. Only minor erroneous allocations in former East Germany (only 5 %) were detected, while more severe erroneous breed assignments in the federal states of former West Germany (91 %) were noted. Ascribing DSN, according to their genetic breed percentage, a rather low inbreeding coefficient of 2 % in recent birth years was discovered. Based on the average increase of inbreeding (0.1 %), the effective population size consisted of 85 animals, confirming the status of German DSN as an endangered cattle population. The relationship between HF and DSN (R= 0.02 %) was negligible. Furthermore, inbreeding depression in production traits was noted for DSN in the first and second lactation. From the extensive DSN pedigree studies, it could be concluded, that there were, and still are, specific breeding strategies in East and West Germany. In East Germany, pure breeding of DSN in large farms was predominant, while in West Germany rather small-scale farms were identified, which often cross bred their DSN with HF, while pure DSN breeding farms were rather scarce. Regarding the inbreeding coefficient and the DSN's endangered state, a monitoring of the breed is strongly recommended as well as the implementation of mating designs, that pay certain attention to inbreeding development.

The second study (**chapter 3**) investigated the usage of innovative trait recording via electronic sensor ear tags, implemented in dual-purpose cattle in pasture-based production systems. Until now, statistical analysis on longitudinal electronic behaviour data has not yet been put into relationship with conventional production or welfare assessment traits (WAT) of dual-purpose cattle, in alternative production systems. The study evaluated interrelations between WAT (body condition score, locomotion score, leg hygiene score, udder hygiene score), temperament traits (general temperament during milking, aggressiveness against other herd members, rank of a cow within herd), longitudinal electronically recorded sensor behaviour traits (rumination, feeding, activity, resting, high activity) and production data (milk yield, fat percent, protein percent) of local DSN on a phenotypic level. Associations, based on phenotypic correlations and

mixed model applications, were used to derive practical management recommendations for farmers, who keep their cows in pasture-based production systems. Regarding subjective WAT and temperament scoring, correlations between the level of aggressiveness towards other herd mates and the intra herd rank order (0.36) were significant, indicating, that cows with a higher intra-herd rank showed increased aggressive behaviour. Mixed model analyses revealed that DSN cows, which spend more time lying down, had reduced daily milk and fat yields. Oppositely, high yielding DSN depicted intensive feeding and rumination behaviour. For the first time, a substantial decrease in rumination and feeding time was observed for cows with elevated somatic cells (>700,000 cells/mL) in pasture systems, suggesting the utilisation of sensor behaviour, as a reliable indicator for udder health. This research has shown, how sensor behaviour patterns were closely related to the production potential of individual cows. Especially, in harsh and extensive environments the subjective scoring of health and welfare traits remains a challenge. Automatically recorded longitudinal behaviour data can be a useful alternative for cow phenotyping in extensive grassland systems. The behavioural observations of this study contributed to a better understanding of dual-purpose cattle physiology. Thus, the gap of understanding cow behaviour in grazing systems could be closed, when combining electronic sensor behaviour, milk production and WAT data. Body condition scoring (BCS) provided sufficiently accurate information regarding the animal's energy reserves. Combined with real time behaviour data, developed models allow the prediction of future BCS, performance levels and contribute to management decisions. Consequently, automatically recorded longitudinal behaviour data is a proper alternative for cow phenotyping, especially to supervise udder health in extensive grassland systems. Another interesting aspect of automated longitudinal cattle behaviour recording is its potential use for the estimation of novel breeding values and genetic parameters. In this regard, cows with favourable behaviour attributes, that are linked to good production and high animal welfare states, can be selected.

The third scientific study (**chapter 4**) based on genomic marker data (i.e. single nucleotide polymorphism: SNP), in order to study functional biodiversity, to conduct a breed assignment of European dual-purpose and dairy cattle breeds and to apply multiple breed GWAS. In the breed diversity sub-study allele-sharing distances (ASD) between individual genotypes were calculated, allowing the prediction of ancestry impact on the

current population structure. The breeds included were Brown Swiss (Sl_BS), Holstein Friesian (Sl_HF) and Simmental (Sl_Si) cattle from Slovenia, Holstein Friesian (PL_HF) and Brown Swiss (PL_BS) cattle from Poland, original dual-purpose Brown Swiss (CH_OBS) and Simmental (CH_Si) cattle from Switzerland and DSN (DE_DSN) and red (DE_DN) cattle from Germany. A principal component analysis (PCA) on the genotype data revealed a clear clustering, depending on the geographic origin of breeds, differentiating between Simmental, Holstein Friesian and Brown Swiss. These three clusters underlined the footprints of artificial selection and the 'genetic isolation' from one another very well. The results of the breed assignment were quite interesting, revealing at least 57.83 % of genetic relations to European cattle breeds, such as Holstein, Hereford, French Red Pied Lowland, Norwegian Red, Red Angus, Bretonne Black Pied, Parthenais, Tarine, Abondance, Charolais, Montbeliarde and French Brown Swiss, affirming a European line of descent. However, exotic ancestral proportions, such as Sheko, Zebu Bororo, Gir or Arabic Zebu were identified within the European genotypes as well. These displayed footprints of exotic breeds are one indication for the evolutionary formative events, that took place during the Neolithic age. They illustrate the migration routes very well, on which breeders spread from the Fertile Crescent towards North-West Europe.

The genetic parameters for behaviour traits were estimated, based on pedigree and genomic relationship matrices. The estimation of genetic parameters (via a combination of genomic relationship matrix and pedigree, as well as only based on pedigree) showed no difference in the outcome of results, ensuring the results' reliability of both methodologies. Genetic parameters for sensor behaviour traits were low to moderate heritable and both variance components, additive-genetic and residuals, were quite high, indicating a potential for selection on dual-purpose cow behaviour. In spite of the small sample size, the standard errors of heritabilities remained acceptable (<0.06), which can be attributed to the plethora of records and the longitudinal data structure of every individual. The highest heritabilities were found for daily high activity (0.19-0.20) and daily feeding (0.19-0.20) behaviour. Sleeping behaviour (0.16-0.18) was under moderate genetic control, while rumination (0.02) and active (0.06-0.08) behaviour as well as ear temperature (0.07) were lowly heritable. The heritability estimations emphasized the opportunity and value for breeders and breeding organizations to select for cows with

desired phenotypic behaviour expressions, that contribute to the animal's wellbeing and physiological sound production levels, as discussed in chapter 3.
Furthermore, the potential of a multi-breed GWAS was investigated, in order to detect significant genomic variants, associated with electronically recorded sensor behaviour data. Genotype and phenotype (sensor behaviour) data from the "2-Org-Cows" project partners (Germany, Poland, Slovenia, Switzerland) was combined to identify significant SNPs, associated with bovine rumination, feeding, active, high active, sleeping behaviour and ear temperature. In spite of the small sample size plausible results were generated. At first, a multi-breed GWAS was conducted on the production trait fat percent. The identification of significant SNPs on chromosome 14, within the region of the *DGAT1* gene, confirmed the reliability of the multi-breed approach. Furthermore, significant SNPs for the traits rumination, activity and feeding were detected on chromosomes 11, 13, 17, 23, 27 and 29. Applying the gene-based test in GCTA, 22 potential candidate genes were discovered and associated with bovine behaviour traits.

In the fourth scientific study (**chapter 5**), quantitative genetic analyses for milk production and novel traits of dual-purpose and HF populations were conducted. Applying a multi-trait animal model (MTAM) allowed the estimation of genetic parameters and correlations within the same trait, to investigate the matter of GxE interactions. In total, eight discrete herd descriptors were chosen, to divide the herd data base into two groups. The chosen herd descriptors for herd grouping considered classical management factors (average herd size, average herd calving age), production characteristics (average herd milk production level, average herd somatic cell count level), genetic descriptors (average percentage of DSN cows within herds, average percentage of natural service sires within herds) and environmental descriptors (altitude and latitude of farm). The study considered 3,659 DSN and 2,324 HF cows from parities one to three. The 46 herds always kept DSN cows, but in most cases, herds were mixed herds (Mixed), including both genetic lines, HF and DSN. Traits were records from the first official test-day after calving for milk yield (Mkg), somatic cell score (SCS) and fat-to-protein ratio (FPR). Genetic parameters were estimated in bivariate runs (separate runs for the three genetic lines Mixed, HF and DSN), defining the same trait from different herd groups or clusters as different traits. For all genetic lines, heritabilities were generally

higher for Mkg, compared to the low heritability traits SCS and FPR. Additive-genetic variances and heritabilities were higher in herd groups indicating superior herd management. This was especially the case for the descriptors: large herds, low calving age, high herd production level and low intra-herd somatic cell count. Herd descriptor group differences in additive-genetic variances for Mkg were most obvious in the HF line, but less pronounced for Mkg in Mixed and DSN. Similar variance components and heritabilities across groups and genetic lines were found for the geographical descriptors altitude and latitude. The residual variances for Mkg were also greater in those herd groups, implying a superior herd management. Permanent environmental variances were close to zero for all traits in all herd groups, due to repeated measurements from different lactations. From 72 bivariate runs, 29 genetic correlations were exactly 1.00 (mostly for Mkg). Somatic cell score was the trait with the lowest genetic correlation, especially for DSN analyses and when stratifying herds according to genetic line compositions (r_g= 0.11) or according to the percentage of natural service sires (r_g= 0.08). Genetic correlations were higher than 0.80 for all traits, when grouping herds according to geographical descriptors. In cluster analyses, genetic correlations were lower than 0.80 for SCS, but close to 1 for Mkg and FPR. The impact of genetic connectedness between herd groups on genetic correlation estimates was assessed as well. However, only a slight trend for larger genetic relationships between groups with increasing r_g estimates was identified.

In **chapter 6**, a relatively new approach was chosen with the deterministic modelling of an organic breeding program design, that accounts for possible GxE interactions. Simulating different breeding plans over a period of 20 years, they were compared, based on calculated annual monetary genetic gain (AMGG), total discounted return (DR) and total discounted costs (DC). The objective was to examine the genetic and economic efficiency as well as implications of specific breeding strategies for a small dual-purpose cattle population, including functional, fertility and production traits. From an economical point of view the organic breeding plan, characterized by natural service sire implementation (NSS), presented a favourable breeding strategy for a small cattle population, emphasizing on functional traits (e.g. body weight) and minimizing costs. Regarding the economic evaluation criteria, the conventional breeding program (usage of test and waiting bull system with artificial insemination: AI), has proven to be the least

suitable approach for a small cattle population. The most desirable economic results, regarding DR, were generated applying the combined breeding plan. The selection scenarios, considering 80-90 % AI and 20-10 % NSS mating in the combined breeding programs, provided a great opportunity, to significantly improve functional traits, such as a reduction in clinical mastitis and an increase in longevity. The essential factors, which affected the economic parameters of breeding programs, were the generation interval, genetic correlations among traits, selection pressure on functional traits, the number of daughters per sire and possible GxE interactions.

In **chapter 7**, a general discussion evaluates the results presented in this study. Furthermore, genetic correlation estimates between production and sensor behaviour traits are investigated. It was shown, that phenotypic and genetic correlations of sensor behaviour and production traits often differed from each other. Mostly, the phenotypic correlations diverged from the genetic correlations between the same traits. The phenotypic correlations between sensor behaviour and production traits remained in a low range (≤ 0.14) with acceptable standard errors. The highest phenotypic correlations were estimated between Mkg and the sensor traits feeding (0.14), high active and sleeping (-0.22) as well as between fat-kg (Fkg) and sleeping (0.13) and Fkg and ear temperature (-0.11). Small correlations were observed between Mkg and rumination (0.07). The estimation of genetic correlations between sensor behaviour and production traits, of different dual-purpose breeds, ranged from low to high values (≥ -0.75 to ≤ 0.65) with rather high standard errors. These can be explained with the rather small sample size and issues of pedigree incompleteness. The phenotypic correlations between sensor and production traits depicted similar trends as the results, generated by the mixed model analyses, which were conducted in chapter 3. It was concluded, that phenotypic correlations among sensor behaviour and between sensor behaviour and production traits were plausible, while the estimation of genetic correlations most likely requires a more extensive data base (greater sample size) as well as a more profound pedigree.

ZUSAMMENFASSUNG

Ziel der Studie war es, eine ausführliche Analyse von innovativen Merkmalen sowie Milchleistungsdaten und damit verbundene Einflüsse von Umweltdeskriptoren auf phänotypischer, quantitativ genetischer und genomischer Ebene am Beispiel des Deutschen Schwarzbunten Niederungsrindes (DSN) in Weideproduktionssystemen durchzuführen. Da bis heute die Zucht von Zweinutzungsrindern auf Daten der konventionellen Leistungsprüfung zurückgreift, können innovative neue Merkmale sowie mittels technischer Automatisierung erfasste Tierwohlindikatoren eine Alternative für moderne züchterische Impulse darstellen. Diese Arbeit beleuchtet das Potential innovative Tierwohl- und Gesundheitsmerkmale als mögliches zukünftiges züchterisches Instrument zur Formulierung neuer Zuchtziele von Zweinutzungsrinderrassen zu nutzen. Die Erfassung von Tiergesundheits- und Tierverhaltensmerkmalen kann künftig dazu beitragen Besonderheiten von lokal angepassten und oftmals vom Aussterben bedrohten Rassen zu identifizieren. Mögliche Besonderheiten bezüglich Robustheit, Fitness, Langlebigkeit und Produktqualität können somit den Erhalt dieser Rassen, im Sinne einer ökologisch ausgerichteten Tierzucht, fördern.

Elektronische Sensoren ermöglichten eine longitudinale Messung tierindividueller Verhaltensparameter (Wiederkäuen, Fressen, Schlafen, Aktivität, gesteigerte Aktivität, Ohrtemperatur) über einen Zeitraum von mehreren Monaten. Neben einer umfangreichen Analyse der Pedigreestrukturen innerhalb der deutschen DSN-Population und Auswertungen zu Populationskennzahlen (Inzucht, effektive Populationsgröße, Verwandtschaft, Inzuchtdepressionen) erfolgten Schätzungen von genetischen Parametern für Produktionsmerkmale unter Berücksichtigung von Genotyp-Umwelt-Interaktionen (GUI). Des Weiteren wurden ökonomische Zuchtplanungssimulationen zur Rentabilitätsbeurteilung verschiedener Zuchtprogramme für Zweinutzungsrassen untersucht. In der vorliegenden Arbeit wurden auf Grundlage unterschiedlicher Datensätze fünf verschiedene wissenschaftliche Studien durchgeführt (siehe **Kapitel 2**, **3**, **4**, **5** und **6**). Die Kapitel behandeln somit unterschiedliche Fragestellungen zur DSN-Rindrinderrasse: 1) Studien zur Inzucht und Verwandtschaft auf Basis eigens berechneter Rasseanteile, 2) Assoziationen zwischen elektronisch erfassten Sensordaten zum Tierverhalten, Milchleistungsdaten und eigens erfassten Gesundheits- und

Tierwohlindikatoren bei DSN-Kühen in Weidehaltung, 3) „Multi-Breed“ genomweite Assoziationsstudie europäischer Rinderrassen zur Identifizierung von Kandidatengenen für bovine Verhaltensweisen, 4) Stratifizierung von Herden nach Umweltdeskriptoren zum Nachweis von Genotyp-Umwelt-Interaktionen, 5) Simulation und ökonomische Bewertung von Zuchtprogrammen unter Berücksichtigung von Produktions- und funktionalen Merkmalen. Abschließend dient **Kapitel 7** der Diskussion der generierten Ergebnisse vor dem Hintergrund der aktuellen züchterischen Entwicklung und gibt einen Ausblick bezüglich zukünftiger Herausforderungen, Trends und Entwicklungsmöglichkeiten in der deutschen DSN-Zucht. Zusätzlich wurden genetische Korrelationen zwischen elektronischen Verhaltens- und Produktionsmerkmalen geschätzt.

In **Kapitel 2** wurde eine intensive populationsgenetische Untersuchung der deutschen DSN-Rasse, auf Basis eigens berechneter DSN-Genanteile, durchgeführt, da die letzten Berechnungen hierzu mehr als ein Jahrzehnt zurückliegen. Unter Berücksichtigung der genetischen Rasseanteile und der offiziellen Auslegung des Fremdgenanteils für DSN wurden in den Kalbejahren von 2005 bis 2016 insgesamt 46 % der Kühe fälschlicherweise der DSN-Rasse zugewiesen. Besonders in den alten Bundesländern waren die Falschzuordnungen mit 91 % sehr hoch und unterschieden sich deutlich von den neuen Bundesländern (5 % Falschzuweisungen). Diese Ergebnisse deuten auf unterschiedliche Zuchtstrategien in Ost- und Westdeutschland hin. So gab es in Westdeutschland wenig „reine“ DSN-Zuchtbetriebe, wobei Anpaarungen von HF-Bullen mit DSN-Kühen sehr häufig waren. Die untersuchten Betriebe in den neuen Bundesländern hingegen verfolgten strikte DSN-Reinzucht und vermieden Kreuzungen zwischen HF und DSN. Basierend auf diesen Erkenntnissen wurden weitere Analysen zur Verwandtschaft, Inzucht, effektiven Populationsgröße, Generationsintervall und Inzuchtdepression, basierend auf der neuen Rassezuordnung, durchgeführt. Generell war der durchschnittliche Inzuchtkoeffizient für den jüngsten Geburtsjahrgang, sowohl bei DSN-Kühen als auch bei DSN-Bullen, mit ca. 2 % niedrig. Ein Inzuchtzuwachs von 0,1 % pro Jahr implizierte eine effektive Populationsgröße von 85 Tieren und hob den Gefährdungsstatus der DSN, als bedrohte Rasse, besonders hervor. Die aktuelle verwandtschaftliche Beziehung zwischen HF und DSN ist mit R= 0,02 % sehr gering.

Inzuchtdepressionen wurden für die Merkmale Milch-kg (Mkg) und Fett-kg (Fkg) in den ersten beiden Laktationen beobachtet.
Trotz der relativ geringen Inzuchtsteigerung pro Jahr sollte sie zukünftig kritisch beobachtet werden. Darüber hinaus ist ein Monitoring der Rasse sowie der Einsatz von Anpaarungsprogrammen, unter Berücksichtigung der Entwicklung von Inzucht und Verwandtschaft, dringend erforderlich.

In **Kapitel 3** wurden Assoziationen zwischen elektronischen Sensordaten zur Tierverhaltensmessung (tägliches Wiederkäuen, Fressen, Schlafen, Aktivitätsverhalten, gesteigertes Aktivitätsverhalten und Ohrtemperatur), phänotypischen Tierwohlindikatoren und Milchleistungsmerkmalen von DSN, aus Weidesystemen, untersucht. Zu den Tierwohlindikatoren gehörten Körperkonditionsbeurteilung, Lahmheitsbefundung, Euterverschmutzung, Beinverschmutzung, allgemeines Temperament während des Melkens, Aggressivität gegenüber anderen Herdengefährtinnen und Rang der Kuh innerhalb der Herde. Korrelationen zwischen den Merkmalen zeigten, dass sich ranghöhere Kühe allgemein aggressiver gegenüber ihren Herdengefährtinnen verhielten (0,36), während rangniedere Tiere in der Regel vermehrte Verschmutzungen an Euter (-0,20) und Hinterbeinen (-0,25) aufwiesen. Mittels linearer gemischter Modelle wurden Zusammenhänge zwischen konventionell erfassten Milchkontroll- und Sensordaten herausgestellt. Kühe mit einem geringeren Milchleistungsniveau verbrachten allgemein mehr Zeit am Tag mit Schlafen, als Kühe mit höherer Milchleistung, die sich durch längere tägliche Futteraufnahme- und Wiederkäuzeiten auszeichneten. Darüber hinaus zeigten Kühe mit gesteigerten somatischen Zellgehalten in der Milch (> 350.000 Zellen/mL) reduzierte tägliche Futteraufnahmezeiten. Basierend auf diesen Ergebnissen konnte der Einsatz des Sensorsystems zur Tierverhaltensmessung bei Zweinutzungsrinderrassen als wertvolles Instrument zur Früherkennung von Euterentzündungen identifiziert werden. Zusätzlich ermöglicht dieser umfangreiche Datenfundus zukünftige rassespezifische Zuchtwertschätzungen für Verhaltensmerkmale. Auf diese Weise können Züchter gezielt Bullen einsetzen, die das Tierverhalten und die Tiergesundheit der Herde verbessern. Dies könnte sich wiederum positiv auf die Betriebsökonomie, in Form von reduzierten Krankheitsinzidenzen (e.g. Mastitis), auswirken. Untersuchungen zum Sensorsystem zeigten, dass praxisrelevante Managementempfehlungen, bezüglich des physiologischen

Verhaltens von Zweinutzungsrassen in Weidesystemen, zuverlässig abgeleitet werden konnten.

Die in **Kapitel 4** aufgeführten genomischen Analysen basierten ebenfalls auf den elektronisch erfassten Sensordaten zum Tierverhalten. Hier sollten mögliche Besonderheiten auf dem bovinen Genom untersucht werden, die mit Verhaltensweisen assoziiert werden könnten. In Form einer „multi-breed“ GWAS wurden Sensordaten von fünf europäischen Rinderrassen aus vier Ländern analysiert. Die Datengrundlage bildeten Genotyp- und Phänotypdaten der Projektpartner des „2-Org-Cows“-Projekts und umfassten Brown Swiss (Sl_BS), Holstein Friesian (Sl_HF) und Simmental (Sl_Si) aus Slowenien, Holstein Friesian (PL_HF) und Brown Swiss (PL_BS) aus Polen, original Brown Swiss (CH_OBS) und Simmental (CH_Si) aus der Schweiz sowie DSN (DE_DSN) und Doppelnutzung Rotvieh (DE_DN) -Kühe aus Deutschland. Zusätzlich diente eine Hauptkomponentenanalyse zur Populationsstratifizierung und ein „Breed Assignment“, basierend auf gemeinsamen Allelen (ASD), zur Untersuchung von Verwandtschaften einzelner Genotypen zu anderen Rinderrassen. Zuletzt wurden genetische Parameter für die elektronisch erfassten Verhaltensmerkmale (Wiederkäuen, Fressen, Aktivität, Schlafen, gesteigerte Aktivität und Ohrtemperatur) auf Basis der genomischen Verwandtschaftsmatrix, in Kombination mit Pedigree sowie nur auf dem Pedigree basierend, geschätzt. Hohe genetische Verwandtschaften (57,83 %) zwischen den genotypisierten Rassen bestanden zu anderen europäischen Rinderrassen, wie Holstein, Hereford, French Red Pied Lowland, Norwegisches Rotvieh, Red Angus, Bretonne Black Pied, Parthenais, Tarine, Abondance, Charolais, Montbeliarde und französisches Brown Swiss. Darüber hinaus zeigten die berechneten ASD auch, dass heutzutage immer noch genetische „Fußabdrücke“ anderer, exotischer Rinderrassen, wie dem Zebu, im Genom der Zweinutzungsrassen zu finden sind. Die Hauptkomponentenanalyse zeigte drei wesentliche Rassecluster, die sich in Holstein, Simmental und Brown Swiss aufteilten. Diese Ergebnisse reflektieren die geografischen Zuchtgebiete der Holsteinlinien, welche überwiegend in Mittel- und Norddeutschland sowie in Polen eingesetzt werden. Im Gegensatz dazu werden in Südeuropa Brown Swiss und Simmental bevorzugt.

Die geschätzten Parameter für Verhaltensmerkmale fielen für beide Schätzmethoden (Pedigree + genomische Verwandtschaftsmatrix, nur Pedigree) sehr ähnlich aus. Die

Verhaltensmerkmale waren gering bis moderat erblich und lagen meist im Bereich bereits geschätzter Erblichkeiten aus anderen Studien. Trotz der geringen Stichprobe waren die Standardfehler mit < 0,06 akzeptabel, was auf das große Datenvolumen und die Vielzahl wiederholter Beobachtungen am Einzeltier zurückgeführt wird. Die höchste Heritabilität wurde für gesteigertes tägliches Aktivitätsverhalten (0,19-0,20) geschätzt. Das Schlafverhalten war moderat erblich (0,16-0,18), wohingegen Wiederkäuen (0,02) und Aktivität (0,06-0,08) sowie Ohrtemperatur (0,07) im niedrigen Erblichkeitsbereich lagen. Anhand der moderaten Erblichkeiten und der z.T. hohen additive-genetischen Varianzen kann man ableiten, dass die elektronisch gemessenen Verhaltensmerkmale durchaus genetisch beeinflusst werden und eine zukünftige Selektion auf besonders vorteilhafte Verhaltensmuster, wie in Kapitel 3 diskutiert, möglich ist.
Zuletzt wurde eine multi-breed GWAS anhand der oben genannten Genotypen, basierend auf den elektronisch aufgezeichneten Verhaltensdaten aus den Ländern Polen, Slowenien, Schweiz und Deutschland, durchgeführt. Aufgrund der geringen Stichprobe wurde die Funktionalität der multi-breed GWAS zunächst für das Produktionsmerkmal Fettprozent, basierend auf konventionellen Testtagsdaten, geprüft. Hier wurden signifikante SNPs auf Chromosom 14 im Bereich des *DGAT1*-Gens identifiziert, was für eine erfolgreiche Anwendung des multi-breed Ansatzes spricht. Die Ergebnisse der multi-breed GWAS zeigten signifikante SNPs auf den Chromosomen 11, 13, 17, 23, 27 und 29, für die Sensormerkmale Wiederkäuen, Aktivität und Futteraufnahme. Nach Anwendung des „gene-based test“ in GCTA wurden insgesamt 22 potenzielle Kandidatengene mit den Verhaltensmerkmalen assoziiert. Es konnte gezeigt werden, dass verlässliche Selektionssignaturen für funktionale innovative Merkmale, trotz geringer Stichprobe (246 Tiere), detektiert wurden.

In der vierten Studie, **in Kapitel 5**, wurde eine quantitativ genetische Analyse (Schätzung genetischer Kovarianzen) für neue und bereits bestehende Merkmale innerhalb von DSN und HF durchgeführt. Aktuell besteht nur bedingt Kenntnis über den Einfluss von Herdendeskriptoren und Umweltfaktoren von Weidesystemen auf die Ausprägung von Produktions- und funktionalen Merkmalen von DSN-Zweinutzungsrindern auf quantitativ genetische (Ko)Varianzkomponenten. Ziel der Untersuchung war daher, das Phänomen von GUI unter Berücksichtigung des „Multiple-Trait Herd Cluster Models“ von Weigel und Rekaya (2000) aufzuklären. Hintergrund dieses „borderless clustering“

ist die Implementierung einer Zuchtwertschätzung über Ländergrenzen hinweg, die nach Charakteristika von Produktionssystemen (Umweltdeskriptoren), anstelle von Ländergrenzen, ausgerichtet ist. Hierbei werden Herden u.a. auf Grund von Managementfaktoren (durchschnittliche Herdengröße, durchschnittliches Kalbealter), geografischer Lage (Breitengrad und Höhenlage des Betriebs), Herdenleistungsniveau (durchschnittliche Milchleistung, durchschnittliche somatische Zellzahl) und genetischen Faktoren (genetischer DSN-Anteil, Anteil Deckbullen im Betrieb) in bestimmte Gruppen (Herdenparameter) eingeteilt. Generell lagen die additiv-genetischen Varianzen, Restvarianzen und Heritabilitäten für Mkg, Log-transformierte Zellzahl (SCS) und Fett-Eiweiß-Quotient für Tiere aus DSN-Betrieben in der von HF-Studien bekannten Bandbreite. Dennoch ergaben sich für die beiden Gruppen innerhalb definierter Herdenparameter teilweise ausgeprägte Differenzen (z.B. Herdengröße). Genetische Unterschiede wurden in größeren Betrieben besser deutlich. Dies wurde auf den Einsatz modernster Managementbedingungen zurückgeführt, die eine bessere Differenzierung des genetischen Potenzials der Tiere bedingten. Zudem waren Erblichkeiten für die Produktionsmerkmale (Mkg= 0,16-0,19) höher als für das Indikatormerkmal der Eutergesundheit (SCS= 0,05-0,12) und den Fett-Eiweiß-Quotienten (0,17-0,09). Die genetischen Korrelationen im gleichen Merkmal zwischen Gruppe 1 und Gruppe 2, innerhalb eines Herdenparameters, lagen im Bereich von 0,08 bis 1,00. Die meisten genetischen Korrelationen übertrafen somit den von Robertson (1959) definierten Richtwert (0,80), welcher als Indikator für eine GUI angesehen wird. Die niedrigsten genetischen Korrelationen ergaben sich für das funktionale Merkmal SCS, insbesondere für die Gruppenbildung nach genetischem DSN-Anteil (0,11) und den Einsatz von Deckbullen in der Herde (0,08). Hohe genetische Korrelationen für Mkg und Fkg deuteten darauf hin, dass auch bei Zweinutzungsrassen in Weidesystemen Leistungsmerkmale mit hoher Heritabilität weniger den Umwelteinflüssen ausgesetzt sind, als Gesundheitsmerkmale mit niedriger Erblichkeit. Verwandtschaftsberechnungen zwischen den Herdenparametergruppen konnten die Hypothese, dass genetische Korrelationen durch Verwandtschaften beeinflusst werden, nicht bestätigen. Obwohl durchaus Indikatoren für GUI nachgewiesen wurden, wird von einer getrennten Zuchtwertschätzung in der DSN-Zucht für unterschiedliche Umwelten abgeraten. Innerhalb Deutschlands werden die Umweltdeskriptoren sich nicht derartig

unterscheiden, als dass ein „Re-Ranking“ der DSN-Zuchtbullen, gemäß unterschiedlichen Umweltbedingungen, notwendig wäre.

In **Kapitel 6** konnte anhand von Zuchtplanungsrechnungen gezeigt werden, dass zielgerichtete Zuchtarbeit bei bedrohten Rassen, die in Weidehaltungssystemen gehalten werden, zu einer Verbesserung funktionaler- und Gesundheitsmerkmale beiträgt. Vor diesem Hintergrund wurde eine eingehende Bewertung von neuen funktionalen Merkmalen in Abhängigkeit von relevanten Umweltdeskriptoren angestrebt. Die ökonomische Bewertung und der züchterische Erfolg wurde an Hand von deterministischen Simulationen unterschiedlicher Zuchtszenarien für eine DSN-Population von 3.000 Tieren verglichen. Vier verschiedene Zuchtprogramme wurden über eine Investitionsdauer von 20 Jahren mit einem Sollzinssatz von 6 % und einem Habenzinssatz von 4 % evaluiert. Das erste Szenario beschrieb ein konventionelles Zuchtprogramm mit Test- und Wartebulleneinsatz, bei 100 % künstlicher Besamung (KB) und Drei-Stufen-Selektion der männlichen Nachzucht. Im zweiten Szenario wurden GUI, basierend auf dem konventionellen Zuchtprogramm, modelliert, indem die genetischen und phänotypischen Korrelationen im selben Merkmal von 0,1 bis 0,9 variierten und auf diese Weise Leistungen von Töchtern in unterschiedlichen Produktionssystemen reflektierten. Das dritte Szenario simulierte ein ökologisches Zuchtprogramm, welches ausschließlich den Einsatz von Natursprungbullen (NSB) und eine Zwei-Stufen-Selektion der männlichen Nachzucht vorsah. Das letzte Zuchtprogramm stellte eine Kombination aus konventionellem und ökologischem Szenario dar, wobei sich der Anteil der männlichen und weiblichen Selektionsgruppen im Bullenpfad änderte. Hier variierte der Anteil der KB und der NSB stetig um 10 %, von 90 % bis auf 10 %, innerhalb des männlichen, des weiblichen, oder in beiden, Selektionspfaden, wobei alle anderen Parameter konstant blieben. Aus ökonomischer Sicht eignete sich besonders das ökologische Zuchtprogramm für kleine Populationen, wie DSN, unter Einbeziehung funktionaler Merkmale. Im Gegensatz zum konventionellen Ansatz fiel der diskontierte Gewinn pro genetische Standardabweichung in den Merkmalen Milchleistung und durchschnittliches Körpergewicht höher aus. Zusätzlich waren die diskontierten Kosten pro Tier im ökologischen Szenario sehr viel geringer (Differenz: 60,43 Euro) im Vergleich zum konventionellen Programm. Dies wurde hauptsächlich auf den Verzicht der Wartebullenhaltung zurückgeführt. Die

Simulation von GUI im konventionellen Zuchtprogramm zeigte, dass sich mit sinkenden genetischen und phänotypischen Korrelationen, im selben Merkmal, der diskontierte Gewinn sowie der jährliche monetäre Zuchtfortschritt reduzierte. Anhand dieser Simulationen konnte gezeigt werden, dass zielgerichtete Zuchtarbeit bei bedrohten Rassen in Weidehaltungssystemen zu einer Verbesserung funktionaler und Gesundheitsmerkmale beitragen kann.

Kapitel 7 fasst in einem allgemeinen Diskussionsteil die wichtigsten Erkenntnisse sowie forschungsrelevante Ergebnisse vor dem Hintergrund der aktuellen Situation der deutschen DSN-Zucht zusammen. Darüber hinaus wird ein Ausblick bezüglich zukünftiger Herausforderungen an- und Trends in der Rinderzucht gegeben. Zusätzlich wurden genetische Korrelationen zwischen Produktions- und Sensormerkmalen geschätzt. Häufig stimmten die phänotypischen und genetischen Korrelationen nicht überein. Die phänotypischen Korrelationen zwischen Sensor- und Produktionsmerkmalen fielen sehr gering aus ($\leq$ 0.14), wobei die höchsten Werte zwischen Mkg und Futteraufnahme (0.14), hoher Aktivität und Schlafen (-0.22) sowie für Fkg und Schlafen (0.13) und Fkg und Ohrtemperatur (-0.11) berechnet wurden. Eine geringere Korrelation wurde zwischen Mkg und Wiederkäuen (0.07) beobachtet. Die geschätzten genetischen Korrelationen zwischen Sensor- und Milchleistungsmerkmalen variierten mit hohen Standardfehlern sehr stark voneinander ($\geq$ -0.75 bis $\leq$ 0.65). Dies wurde auf die geringe Stichprobenzahl und Pedigreeunvollständigkeit zurückgeführt. Folglich ergaben sich sehr plausible Werte für die phänotypischen Korrelationen, wohingegen die genetischen Korrelationen kritisch zu betrachten sind.

CHAPTER 1

General Introduction

Changes in the dairy sector

Over the last decades, the European dairy sector has undergone several structural changes as a result of dynamic adaptation processes along with altering ways of production (Huettel and Jongeneel, 2008). One of these trends is the increasing momentum of (organic) pasture-based milk production (Rosati and Aumaitre, 2004). Organic dairy production is the fastest growing organic food segment in the United States (DuPuis et al., 2000). Correspondingly, the EU organic dairy production has nearly doubled since 2006 and stands at 3.8 million metric tons, representing more than 2.6 % of the EU's milk production in 2014. While the organic farm land in Germany constituted of 6.3 % of the total land share in 2014 (IFOAM, EU and FiBL 2016). Driven by ethical and moral values (e.g. commitment to locality, ecological sustainability and animal stewardship considerations), consumer requests for safer and more sustainably produced food are growing (Thongplew et al., 2016; Conner et al., 2008). As a consequence, companies, such as Danone and FrieslandCampina, engaged in organic food provision, due to profitable market opportunities and the aspect of corporate social responsibility (Maloni and Brown, 2006; Thongplew et al., 2014).

In this regard, Hambrusch et al. (2011) discussed the challenge of the German dairy industry, to increase the added value of their products and thusly assuring long term business success. Their analysis about the consumer's willingness-to-pay revealed, that there is a potential market for pasture-based dairy products and a common desire for food to be 're-embedded' in nature. However, legally binding guidelines for dairy products, generated from pasture-based production systems, do not exist (Hambrusch et al., 2012). Attributes, such as health, animal welfare and environmental issues, which are highly valued by the consumer, are associated with pasture-based dairy production (Hambrusch et al., 2012). For that matter, a widely discussed aspect is the beneficial effect of higher concentrations of conjugated linoleic acid and unsaturated fatty acids in the milk, which result from pasture-grazed cows (Croissant et al., 2007). Next to the 'natural image' of pasture-based production and the putative health effects, that are presumed to come with it, the system offers environmental benefits compared to row crop production. Accordingly, a reduction of sediment erosion, phosphorus runoff and improved carbon sequestration only constitute a few ecological benefits (Conner et al., 2007).

Nonetheless, literature has shown, that the organic production strategy does not automatically meet the demands for animal health and welfare, as these attributes conflict with high productivity and low costs (Sundrum, 2001). Decreasing the feed intake level in high genetic merit milking cows could entail negative effects on health and reproduction (Sehested et al., 2003). Concerning this matter, the question of which dairy cow breed is best suited for the special challenges of pasture-based production will be closer examined.

Grassland systems and organic dairy production

Since the late 1960's the awareness, that highly specialized breeds gain more profit than a single breed, selected for various different traits, strongly influenced dairy cattle breeding. This contributed to the so called 'holsteinization' phenomenon and a consistent implementation of North American bull sires worldwide (VanRaven, 2004; Harris and Kolver, 2001, Philipsson and Lindhé, 2003). Relating to this, the selection for tall, high yielding dairy cows was favoured, as bigger heifers and cows generated higher sales prices on the market. At this point in the past, little attention was paid to health and fitness traits as well as to the steady fertility decline in dairy cattle. Furthermore, the aspect of increasing feed requirements of bigger cows was often unnoted (VanRaven, 2004). However, the issue of feed costs should not be handled inadvertently, as they present a fundamental economic part of animal production (Rauw et al., 1998).

As a consequence of fluctuating milk prices and increasing labour, machinery, housing and feeding costs, a resurgence of interest in pastoral dairy farming was observed in New Zealand, Australia and parts of western Europe (Macdonal et al., 2008). Although conventional dairy farming depicted higher production levels than pasture-based systems, White et al. (2002) reported lower operating expenses and higher net incomes per cow in pasture systems. Including grains and concentrates into the diet of dairy cattle in New Zealand has shown to be 6 to 12 times as expensive as grazing. This has caused New Zealand farmers to adopt low-cost production technologies, such as year-round grazing and strict seasonal calving, which is especially suitable in temperate regions (Rinehart, 2009). As a corollary and due to the homogenous structure of the New Zealand milk producing system, certain breeding goals have been pursued over 50 years, which focus on functional traits (e.g. claw health, disease resistance), high fertility, longevity/ robustness and efficient production of milk solids (Barth et al., 2004). In other words, the

New Zealand merit index favours resilient, lightweight, long living and efficient milk producing pasture converters (Mertens et al., 2011). The strong selection for these breeding characteristics in New Zealand resulted into dairy cattle with increased longevity (6-7 years) and parities (4.6 lactations), reduced average calving intervals (368 days), low lameness and mastitis incidences and a 'digestion type', that efficiently covers its energy demand solely by feeding on roughage (Barth et al., 2004). When Harris and Kolver (2001) compared the performance between North American Holsteins and New Zealand Holstein cattle, the lighter New Zealand Holsteins revealed lower milk yields with higher concentrations of fat and protein. Moreover, the New Zealand line expressed a high level of fertility and robustness, while the American Holstein cows depicted low fertility rates. Overall, the analysis revealed an average advantage of $NZ 4,950 per farm per year in favour of New Zealand cows, which corresponded to approximately 12 % difference in economic farm surplus (Harris and Kolver, 2001).

The example of pastoral dairy production in New Zealand has demonstrated the variety of dairy cattle farming. Although many breeding programs are global, farmers and breeders can benefit from implementing and selecting unconventional breeds, which match local markets or are better adapted to certain environments and purposes (VanRaden, 2004). As described earlier, the rejuvenated interest in organic farming constitutes a classic example. Similar to year-round pasture production, organic dairy farming has to meet different management needs than conventional production does and is affected by environmental influences more severely (Rosati and Aumaitre, 2004). Consequently, the choice of which breeds to implement and which breeding goals to focus on is fundamental. The principle of organic farming obliges the adaptation of an animal towards the local and natural production system, rather than the adaption of the environment towards the requirements of high-yielding breeds. Apart from that, European organic production standards strictly limit the prophylactic use of antibiotics, which prevents dairy farmers from routinely treating their cows with antibiotics, to reduce the risk of mastitis infections. Aside from medication restrictions, forage-based feeding (60 % of daily dry matter intake) and primarily home-grown diets, along with restrained supplement feeding are stipulated. These factors greatly affect the physiology of milking cows. Due to less energy enriched feeds cows are expected to have lower milk yields,

while breeds of high genetic merit are at increased risk to suffer from energy deficiency, resulting into metabolic disorders and poor fertility (Hovi et al., 2003).

A Swedish survey revealed, that organic farmers desired a higher genetic gain of their herds in disease resistance (mastitis and parasites), rather than in milk production (Ahlman et al., 2014). Ahlman et al. (2014) have shown, that the farm management type (organic vs. conventional) was the most important criteria, influencing the dairy farmer's preference of traits. Hence, functional traits (e.g. reproduction, health, longevity) were of greater importance for organic dairy farmers than for conventional milk producers. On the other side, conventional dairy producers valued milk production more than organic farmers (Ahlman et al., 2014). This suggests, that due to different breeding goal preferences, organic farmers might favour the implementation of alternative cow genetics, which are better suited for extensive systems. In this regard, Sundberg et al. (2009) pointed out, that crossbreeding and local breeds, such as Swedish Red, were more common in Sweden in organic production compared to conventional farming. This concurs with the results of Dillon et al. (2003), who compared French dual-purpose cows (Montbeliarde) to North American Holsteins in a seasonal grass-based system. They pointed out the Montbeliardes' outstanding potential for preferable milk composition (especially omega-3 fatty acids and conjugated linoleic acids), higher meat merit (highly marbled meat) and increased pregnancy rates. Additionally, they recorded greater herbage-concentrate substitution rates and higher herbage dry matter intake per kg live weight, which emphasized their ability to produce milk from herbage alone (Dillon et al., 2003).

Therefore, it is hypothesized, that local dual-purpose breeds, that adapted to harsh environments over the course of many years, are better suited for organic milk production than high producing cattle breeds, which are predominantly kept in conventional indoor systems.

Situation of endangered native dual-purpose cattle breeds in Germany

Aside the adaptation of indigenous breeds to specific habitats, there are other substantial reasons for implementing and maintaining local landraces in extensive production systems. These breeds, which are often specialized to live in one certain environment and display a high degree of resilience, represent unique genetic resources, which might be valuable in the future. As these traits have co-evolved with a particular environment or

husbandry system, they cannot simply be re-established. The Food and Agricultural Organization (FAO) described the maintenance of genetic diversity as an insurance package against adverse future conditions (Brem et al., 1989). Moreover, indigenous breeds are considered a genetic reservoir, in case effective and utilizable genetic variation in the predominant population decreases. Additionally, the value of cultural heritage of local domestic breeds has to be considered. In certain regions, these breeds contribute to local colour, recreational value and play a significant role in tourism (Brem et al., 1989). Medugorac et al. (2009) emphasized the importance of conserving genetic diversity by preserving (endangered) native cattle breeds.

Of the 1,478 officially world-wide recorded cattle breeds, 254 have already become extinct (Taberlet et al., 2008; Medugorac et al., 2009). To this effect, it is fundamental to encourage the implementation and maintenance of local endangered breeds in extensively/organically managed farms or for landscape conservation purposes, through state subsidy, in the form of research projects, pilot and demonstration projects, zoos or cryopreservation (Barth et al., 2004). Unfortunately, there are no legal means, which prevent a further depletion of genetic diversity (Barth et al., 2004). So far, only the EU regulations 1804/1999 regarding organic animal production stipulated, that "in the choice of breeds, account must be taken of the capacity of animals to adapt to local conditions […] Preference is to be given to indigenous breeds and strains." Nonetheless, Barth et al. (2004) concluded, that endangered cattle breeds were often kept together with conventional breeds in organic farms. In many cases, farmers did not entirely renounce of high merit dairy cows, as they accounted for the economic basis of these farms, due to their higher production level.

In 2003, the ministry of Food and Agriculture and Consumer Protection appointed a National Committee on Animal Genetic Resources to monitor the state of German farm animals and release early warnings (red list) for breeds at risk (TGRDEU). The basis for categorizing the (endangerment) state of a population is the effective population size, which is calculated on herd-book numbers. According to the red list, there are 27 indigenous cattle breeds in Germany, of which 20 are at risk of becoming extinct (TGRDEU). Figure 1 gives an overview about endangered indigenous cattle breeds, the number of males and females and their effective population size (N_e), which were listed in the database of the 'Central Documentation on Animal Genetic Resources in Germany'

in 2017 (TGRDEU). Five of the depicted breeds were additionally listed as beef lines (Gelbvieh, Hinterwälder, Pinzgauer, Vorderwälder, Murnau-Werdenfelser), which are not presented in the graphic. The national program of the TGRDEU differentiates between different risk categories: phenotypic conservation population ($N_e \leq 50$), conservation population ($N_e \leq 200$), monitoring population ($200 < N_e \leq 1000$) and non-endangered population ($N_e > 1000$). In some cases, such as the old Angeln cattle breed, the severity of the breed's actual state of endangerment, was recognized very late (Barth et al., 2004), which complicated the implementation of breeding programs and further conservation measures. Referring to N_e, rather serious situations are also depicted for Pinzgauer, Murnau-Werdenfelser, Gelbvieh, Doppelnutzung Rotbunt and Ansbach-Triesdorfer. According to the TGRDEU, breeds with an effective population size ≤ 50 only stand a small chance of becoming an independent live population again. It is recommended to secure their existing genetic stock by means of cryoconservation. Furthermore, they could be integrated into larger related populations.

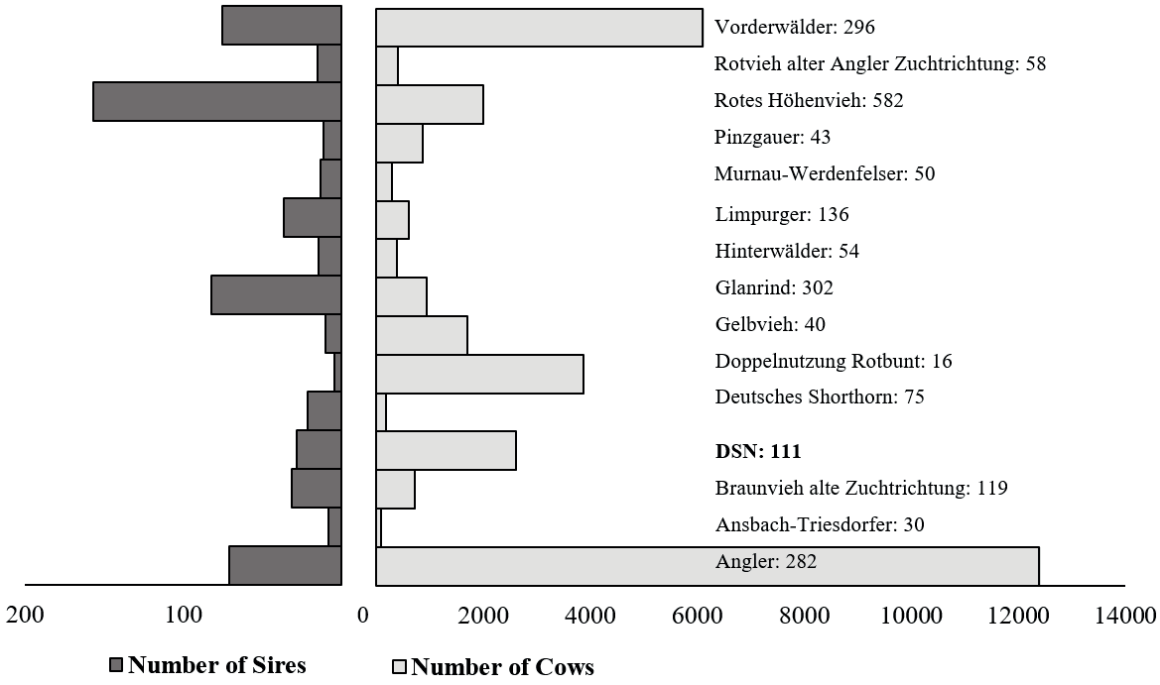

***Figure 1**: Number of male and female cattle breeds and their effective population size, in 2017, listed in the 'Central Documentation on Animal Genetic Resources'. The effective population size (N_e) is calculated based on the herd-book data as documented in TGRDEU according to the following formula: (4 x males x females) / (males + females).*

In order to engage in breeding activities, a certain population size and genetic variability is required as a basis for selection, while maintaining a minimum inbreeding level. Particularly, in regard to preserving fitness and genetic adaptability, Soulé (1980) recommended an N_e of 500. Shaffer (1981) reasoned, that a minimum viable population is not one, that can maintain itself under average conditions, but one, that is of sufficient size to endure the calamities of various perturbations (e.g. diseases, environmental catastrophes). In the case of the old Angeln breed, at least 13 sires and 184 dams were considered necessary to maintain the population without high inbreeding rates (Barth et al., 2004). For this reason, the DSN population was chosen for further investigation, as a sufficient genetic diversity and (effective) population size was given.

In this regard, within the course of the '2-Org-Cows' project, the study examines the suitability of dual-purpose DSN cows, kept in pasture-based production systems, based on innovative trait comparisons on a phenotypic, genetic and genomic level. Aside from milk production data, health trait and welfare indicators were recorded in the German black and white dual-purpose breed, in order to assess their robustness and identify potential candidate genes, that might support a better adaptation towards harsh environments. Moreover, economic evaluations, based on deterministic simulations, were applied, to find the optimum breeding approach for a small local dual-purpose breed. All these aspects are addressed in separate chapters, that recursively interdepend on each other.

The **2nd chapter** outlines the actual situation of DSN breeding in Germany, conducting a thorough analysis of population parameters (inbreeding coefficient, average relationship, effective population size, influential sires). An own algorithm is programmed to assure the genetic breed percentage of DSN. Based on these newly calculated breed percentages, a linear mixed model is applied, using official DSN test-day data, to check for potential inbreeding depressions.

The **3rd chapter** investigates the implementation of a new herd management tool in a pasture-based production system. So far, the electronic sensor technology was only used in indoor housing systems and predominantly applied in milking breeds. Phenotypic correlations and linear mixed model applications are used to study the relations between the electronically recorded data with subjectively scored health and welfare traits as well as milk production records and to derive possible management recommendations.

Chapter 4 explores the electronically recorded sensor traits on a quantitative genetic and genomic level. A PCA is conducted for population stratification purposes, while ASD are calculated to assess genetic relationships between DSN and other European cattle breeds. Furthermore, genetic parameters are estimated as well as genomic significant variants are studied, applying a multi-breed GWAS.

The **5th chapter** investigates whether German DSN herds are under the influence of GxE interactions. Consequently, herds are grouped according to distinct environmental descriptors, following the approach of Weigel and Rekaya (2000). Implementing a multi-trait animal model, genetic parameters of test-day data are estimated separately within the same trait (Weigel and Rekaya, 2000). Due to the different farm management situations in East and West Germany (see chapter 2), exploring the matter of GxE interactions is important, to clarify whether implementing separate breeding value estimation for DSN or re-ranking breeding sires is required.

The **6th chapter** compares different deterministic breeding plan simulations for a small DSN population, including functional traits in the breeding goals and considering GxE interactions. Several schemes of conventional (based on AI), organic (based on NSS) and combined breeding of DSN cattle are compared, to optimize annual monetary genetic gain, discounted return while minimizing discounted costs.

In **chapter 7**, most important results of the previous chapters are discussed against the background of current and future challenges of dairy cattle breeding. In this regard genetic correlations are estimated between production and sensor traits, exploring the relations between economically important traits and animal behaviour on a quantitative genetic scale. Based on relevant findings, recommendations for organic breeders and farmers are given, using the example of DSN, to address the more than ever pressing and actual topic of the future direction sustainable (dual-purpose) cattle breeding should take.

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CHAPTER 2

Inbreeding and Genetic Relationships of the Endangered Dual-Purpose Black and White Cattle Breed Based on Own Genetic Breed Percentage Calculations

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Einleitung

Das „alte“ Deutsche Schwarzbunte Niederungsrind (DSN) hat seinen züchterischen Ursprung in den Marsch- und Niederungsgebieten im Nordsee-Raum. Wegen seiner Anpassungsfähigkeit, fruchtbaren und futterdankbaren Eigenschaften bei gleichzeitig hoher Dauerleistung an fettreicher Milch und guter Mastfähigkeit, wurden und werden DSN-Rinder vielseitig in Deutschland und den Niederlanden eingesetzt (Gassan, 2017; Brade und Brade, 2013). Die Zuchtgeschichte der DSN wurde intensiv von Mügge et al. (1999) betrachtet. Dazu gehören insbesondere im Kontext der Rasseentwicklung die unterschiedlichen züchterischen Strategien unter verschiedenen Haltungsbedingungen seit Anfang der 60iger Jahre im Ost- und im Westteil Deutschlands. In Ostdeutschland wurden DSN mit Jerseys und Holstein Friesian (HF) gekreuzt, was zur systematischen Konsolidierung der neuen synthetischen Rasse „Schwarzbuntes Milchrind“ (SMR) führte. Zeitgleich wurden Ende der 60iger Jahre sieben Stammzuchtzentren für DSN eingerichtet, unter anderem Gut Kölsa im Bezirk Cottbus, Lebusa und Gräfendorf. Der Bestand verfügte insgesamt über 4.000 schwarzbunte Kühe, 100.000 eingelagerte Spermaportionen und jährlich 15 bis 20 zur Zucht aufgestellte Jungbullen. In Westdeutschland führte die Verdrängungskreuzung mit HF zu grundlegenden Änderungen des Rassecharakters der Schwarzbuntpopulation. Als Reaktion auf die fortlaufende Dezimierung der DSN-Bestände gründete sich 1989 der „Verein zur Erhaltung und Förderung des alten schwarzbunten Niederungsrindes“ (VEF-DSN), der in Westdeutschland Aufgaben der Zuchtorganisation und Herdbuchführung übernimmt. Zusätzlich wurde in Mariensee eine Genreserve mit Embryonen und Sperma angelegt. Nach der Wiedervereinigung wurde die Zuchtarbeit der DSN-Rasse in den neuen Bundesländern durch den Rinderzuchtverband Berlin-Brandenburg eG organisiert (RBB). Von der Landesregierung gewährte Fördermittel ermöglichten die fortbestehende Erhaltung der kulturhistorischen Genreserve. In den alten Bundesländern ist weiterhin der VEF-DSN aktiv, um einen deutlichen Beitrag zur Konsolidierung der Rasse als auch zu deren züchterische Weiterentwicklung zu leisten. Erst im Jahre 1997 erfolgte eine strikte Trennung von DSN und HF durch die Vergabe getrennter Rassecodes (RC; DHV, 2017). Die DSN erhielten RC= 10 und HF der Farbrichtung Schwarzbunt wurde RC= 01 zugewiesen.

Trotz der Aktivitäten des RBB und des VEF-DSN werden DSN-Bullen heutzutage nur eingeschränkt und meist in Betrieben mit hohem Grundfutteranteil in der Fütterung eingesetzt, da sie aufgrund geringerer Milchmengenzuchtwerte nicht mit Hochleistungsrassen konkurrieren können. Biedermann (2003) berichtete, dass 47 % der DSN-Tiere in ökologisch wirtschaftenden Betrieben gehalten werden. Das Potenzial der Rasse DSN liegt in diesem Zusammenhang in ihrer langen Nutzungsdauer und Gesundheit sowie Anpassungsfähigkeit an extensivere Haltungsbedingungen, wie sie beispielsweise in der stark weidebasierten Milchproduktion anzutreffen sind (Biedermann, 2003).

Trotz dieser Vorzüge für DSN handelt es sich um eine gegenwärtig kleine Population mit nur 2.847 (Stand 2016) Herdbuchkühen (TGRDEU). Inzuchtmanagement und die Erhaltung genetischer Eigenständigkeit bzw. Diversität stellen die Kernelemente eines Erhaltungszuchtprogrammes in kleinen lokalen Rassen dar (Hartwig und Bennewitz, 2014). Die Erhaltung des robusten Rassecharakters der DSN bzw. die Realisierung von Zuchtfortschritt, insbesondere in den niedrig erblichen funktionalen Merkmalen, stellt eine große Herausforderung dar, da für Fitnessmerkmale Inzuchtdepressionen zu erwarten sind (DeRose und Roff, 1999). Biedermann et al. (2005) berechneten einen Inzuchtkoeffizienten von 2,5 % mit einer Inzuchtsteigerung von 1,4 % pro Generation in 2003 für die DSN-Gesamtpopulation unter Einbeziehung der fünf zurückliegenden Generationen, ohne konkret die weiteren Auswirkungen von Inzucht zu analysieren.

Ziel dieses Beitrages ist es, nach nun mehr als 15 Jahren eine umfassende populationsgenetische Analyse innerhalb der DSN-Rasse durchzuführen. Da vorherige DSN-Studien zeigten, dass genetische Rasseanteile von DSN-Tieren nicht zwangsläufig den eingetragenen Rassecode widerspiegeln (Biedermann et al. 2005; Ehling et al. 1999), wurde ein eigener Algorithmus entwickelt, um DSN-Genanteile zu berechnen. Sämtliche Auswertungen zur Inzucht, Verwandtschaft, weiteren Determinanten des Zuchtfortschritts als auch zu etwaigen Inzuchtdepressionen basieren somit auf den eigenständig berechneten Genanteilen bzw. daran anschließenden Rasseeinteilungen.

Material und Methoden

Datenmaterial

Die vom VIT (Vereinigte Informationssysteme Tierhaltung e.V., Verden) bereitgestellte Abstammungsdatei für DSN-Tiere und Herdengefährtinnen anderer Rassen beinhaltete insgesamt 82.538 Tiere. Die Zuordnung der Tiere zu DSN bzw. HF basierte auf den Ergebnissen des eigens entwickelten Algorithmus. Für die Zuordnung eines Tieres zu DSN wurden mindestens 90 % DSN-Genanteile verlangt (DSN_90 %) und dementsprechend für die Zuordnung eines Tieres zu HF 90 % HF-Genanteile vorgegeben (HF_90 %).

Die Verwandtschaftsberechnungen zwischen aktiven DSN_90 %-Kühen mit definierten Subgruppen nach Leistungsniveaus oder geographischer Zuordnung sowie zu einflussreichen Vätern basierten auf der Datengrundlage des Kalbejahres 2015 und 46 Betrieben (davon 44 Betriebe der alten Bundesländer mit mindestens fünf aktiven DSN Kühen). Dabei wurden Untersuchungen nur innerhalb der ersten Laktation durchgeführt (238 DSN_90 % Kühe) als auch unter gemeinsamer Berücksichtigung von Kühen der ersten bis dritten Laktation (573 DSN_90 % Kühe). Zur Berechnung von verwandtschaftlichen Beziehungen zwischen DSN-Kühen und HF-Kühen wurde die Restriktion für den DSN-Anteil in den Herden außen vorgelassen und somit 928 DSN-Kühe und 1.190 HF-Kühe des Kalbejahres 2015 und den Laktationen eins bis drei in die Analysen inkludiert. Für die Untersuchungen zu der Auswirkung von Inzucht in der DSN_90 %-Kuhpopulation und der HF_90 %-Kuhpopulation wurden die Kalbejahre 2005 bis 2017 berücksichtigt. Das Interesse lag dabei auf dem Studium der Effekte von Inzucht in der Frühlaktation. Somit wurden die Merkmale Milch-kg (Mkg), Fett-kg (Fkg) und log-transformierte Zellzahl (SCS= log 2 (Zellzahl/100.000) + 3) vom ersten offiziellen Testtag einbezogen.

Algorithmus zur Berechnung von DSN-Rasseanteilen

Die Berechnung der tierindividuellen Rasseanteile (RA) erfolgte iterativ ausgehend von den Gründertieren (Generation 0 ohne bekannte Vorfahren) über alle Generationen hinweg im gesamten Pedigree (Abbildung 2).

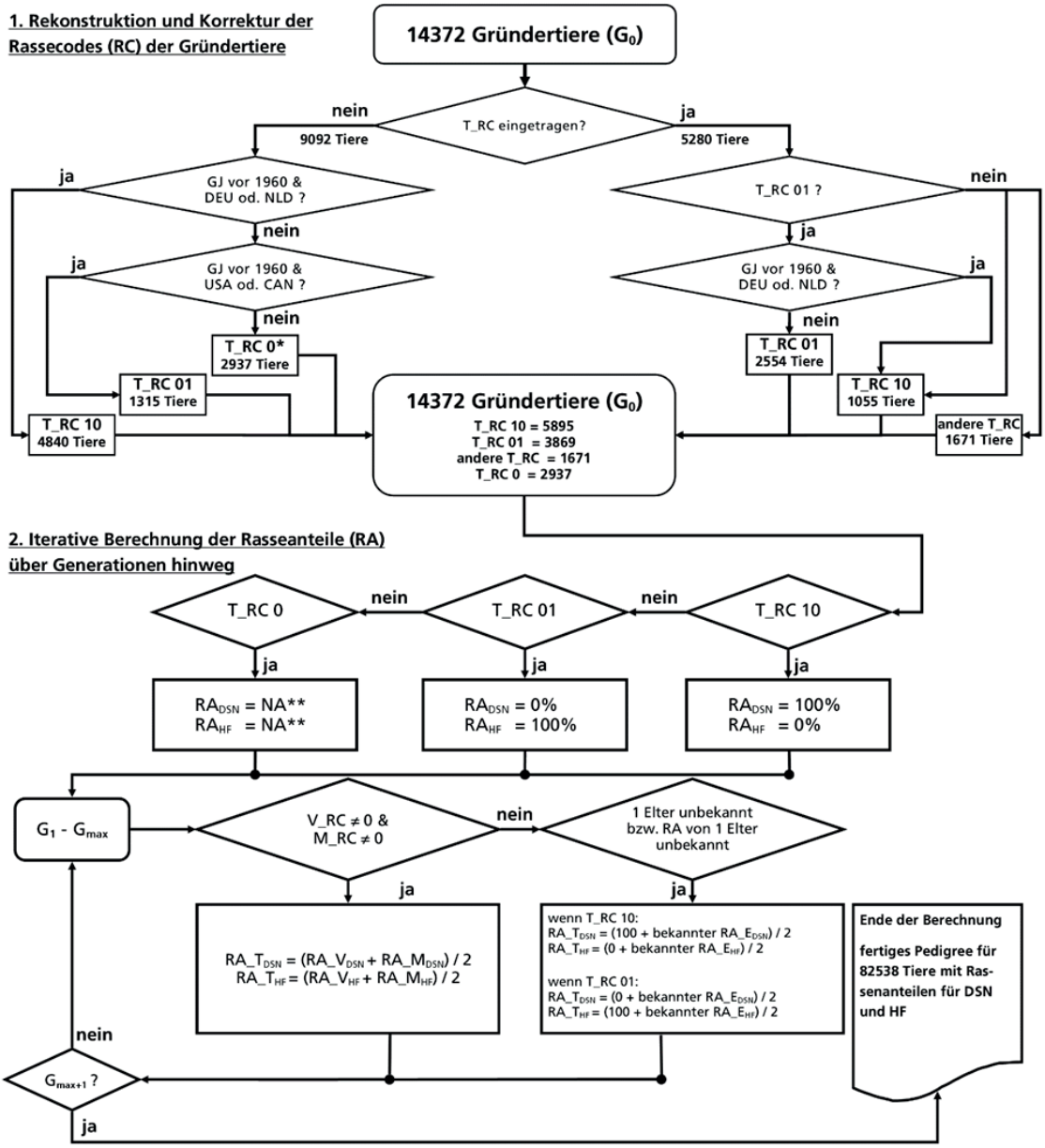

***Abbildung 2**: Flowchartdiagramm des Algorithmus zur Berechnung der Rasseanteile für DSN und HF im Pedigree. *RC 0= entspricht einem unbekannten Rassecode (RC), ** NA= fehlende Werte, T= Einzeltier, V= Vater, M= Mutter, E= Eltern, G= Generation, GJ= Geburtsjahr, RA= Rasseanteil.*
*Flowchart diagram illustrating the algorithm to calculate breed percentages of DSN and HF in pedigree data. * RC 0= reflects an unknown breed code (RC), ** NA= missing value, T= animal, V= sire, M= dam, E= parents, G= generation, GJ= birth year, RA= breed percentage.*

Bei Gründertieren liegt nur dann Information zum Rassecode vor, wenn dieser auch eingetragen ist. Für die Gründertiere lassen sich dennoch Kriterien definieren, die eine klare Zuordnung zur Rasse DSN erlauben: 1. Geburtsjahr vor 1960, 2. entsprechend seiner Lebensohrmarke ist das betreffende Tier als deutsches oder niederländisches Tier registriert (d.h. ISO-Ländercode 276 für Deutschland oder 572 für die Niederlande) und

3. das betreffende Tier hat entsprechend der ISO-Ländercodes ausschließlich deutsche Nachkommen. Von 9.092 Gründertieren ohne eingetragenen Rassecode konnten auf diesem Weg 4.840 Tiere eindeutig der Rasse DSN zugeordnet werden. Darüber hinaus wurden, basierend auf den genannten Kriterien, 536 als HF mit RC= 01 registrierte Gründertiere, als eindeutige DSN Tiere identifiziert. Deren Rassecodes wurden entsprechend zu RC= 10 umcodiert.

Die initiale Berechnung der Rasseanteile für DSN und HF bei den Gründertieren erfolgte im ersten Schritt auf Basis der wie zuvor beschrieben rekonstruierten oder korrigierten Rassecodes. So wurde dementsprechend Gründertieren mit RC= 10 ein DSN-Anteil von 100 % zugewiesen. Analog erhielten Tiere mit RC= 01 oder RC= 02 (HF-Farbrichtung Rotbunt) einen HF-Anteil von 100 %. Für Gründertiere, deren Rassecode auch nach der oben beschriebenen Prüfung unbekannt war, wurden keine Anteile berechnet. Die Berechnung der Rasseanteile in Prozent ab Generation 1 erfolgte für den Fall, dass beide Elterntiere bekannt waren, mit folgender Formel:

$$RA_{Nachkomme} = (RA_{Vater} + RA_{Mutter}) / 2 .$$

War eines der Elterntiere unbekannt (d.h. eine vorherige Berechnung der Rasseanteile für das unbekannte Elterntier, wie oben beschrieben, war nicht möglich), so wurde der registrierte RC des Nachkommen als Referenz verwendet. Dies impliziert für ein Tier mit RC = 10, dass der DSN-Rasseanteil wie folgt berechnet wurde:

$$DSN\text{-}RA_{Nachkomme} = (100 + DSN\text{-}RA_{bekannter\ Elternteil}) / 2 .$$

Analog wurde für HF vorgegangen. Der iterative Algorithmus, inklusive der Ableitung und Korrektur, der Rassecodes der Gründertiere wurde im Software-Paket R (R Core Team, 2017) eigenständig programmiert. Die Berechnung der tierindividuellen Generationenzahl (die maximale Generationenzahl für Einzeltiere betrug 25 Generationen) erfolgte mit dem R-Paket „pedigree V1.4“ (Coster, 2012) vor der Berechnung der Rasseanteile.

Tabelle 1 zeigt die Zuordnung der Kühe nach berechneten Rasseanteilen innerhalb der alten und neuen Bundesländer. Die Berechnungen der Rasseanteile ergaben in den Kalbejahren von 2005 bis 2016 für insgesamt 46 % der DSN-Tiere und für 22 % der HF-Tiere Zuordnungen, die nicht mit der ursprünglichen Rassecodierung in Einklang waren.

Aus diesem Grund basieren alle folgenden Berechnungen auf der Einteilung der Tiere gemäß ihren genetischen Rasseanteilen an Stelle von offiziellen Rassecodierung.

Tabelle 1: Rasseanteile innerhalb der Rassecode-Zuordnungen (DSN, HF) der Kühe für Kalbejahre 2005-2016 und gesondert für 2015 innerhalb der alten (ABL) und der neuen Bundesländer (NBL)
Genetic breed percentage of cows with assigned breed codes for DSN and HF for calving years 2005-2016 and for the calving year 2015, stratified by federal states of former West Germany (ABL) and East Germany (NBL).

Rassecode	Rasseanteile	Kalbejahre 2005-2016			Kalbejahr 2015		
		Gesamt	NBL	ABL	Gesamt	NBL	ABL
DSN= 10	< 90 % DSN	3.406	187	3.219	1.005	81	924
	≥ 90 % DSN	4.074	3.749	325	928	806	122
HF= 01	< 90 % HF	1.703	37	1.666	470	9	461
	≥ 90 % HF	6.141	2	6.139	1.190	0	1.190

Verwandtschaft, Inzucht und effektive Populationsgröße

Für die Analyse von durchschnittlichen Verwandtschaften (R) innerhalb und zwischen definierten Gruppen der DSN_90 % wurde das Programmpaket CFC (Sargolzaei et al., 2006) verwendet. Der Inzuchtkoeffizient (F) wurde nach Wright und McPhee (1925) ebenfalls in CFC berechnet. Die mittleren Inzuchtkoeffizienten pro Geburtsjahr für DSN_90 %-Bullen und Kühe wurden getrennt berechnet. Die effektive Populationsgröße (N_e), basierend auf der Inzuchtentwicklung nach Falconer und Mackay (1996), wurde aus der Inzuchtsteigerung pro Generation abgeleitet:

$$N_e = \frac{1}{2 \times \Delta F}.$$

Zur Berechnung des Inzuchtanstiegs pro Generation (ΔF) wurden mittlere Inzuchtkoeffizienten innerhalb der Geburtsjahrgänge der DSN_90 %-Population berechnet und die Zusammenhänge zwischen Geburtsjahr und Inzuchtkoeffizient mittels linearer Regression abgebildet. Die hieraus resultierende Inzuchtsteigerung pro Jahr wurde mit dem Generationsintervall multipliziert. Nach Falconer und Mackkay (1996) ist das Generationsintervall das durchschnittliche Alter der Eltern bei Geburt ihrer zur Zucht verwendeten Nachkommen. Daher wurde die Differenz des Alters der Kühe des Geburtsjahrgangs 2011 zum Geburtsjahrgang ihrer zur Zucht verwendeten Töchter bestimmt und mit der durchschnittlichen Inzuchtsteigerung pro Jahr multipliziert.

Auswirkungen von Inzucht

Es wurden für alle Merkmale getrennte Rechenläufe innerhalb DSN_90 % und innerhalb HF_90 % durchgeführt. Tabelle 2 zeigt die deskriptive Statistik der verwendeten

Leistungsmerkmale Mkg, Fkg und SCS am ersten Testtag der Laktationen eins bis drei für DSN_90 % und HF_90 %.

Tabelle 2: Deskriptive Statistik der Leistungsmerkmale Milch-kg (Mkg), Fett-kg (Fkg) und log-transformierte Zellzahl (SCS) vom ersten Testtag der Laktationen eins bis drei für DSN_90 % und HF_90 %.
Descriptive statistics for production traits milk (Mkg), fat (Fkg), and somatic cell score (SCS) from the first test-day in parities one to three for DSN_90 % and HF_90 %.

Gruppe	Laktation	Merkmal	N	Mittelwert	SD	Min	Max
DSN_90 %	1	Mkg	3.286	23,56	5,32	2,00	53,80
	2	Mkg	2.644	33,23	6,78	2,00	50,90
	3	Mkg	2.032	34,76	7,49	2,00	55,60
	1	Fkg	3.286	0,96	0,24	0,09	2,55
	2	Fkg	2.644	1,42	0,35	0,11	3,05
	3	Fkg	2.032	1,53	0,38	0,08	3,08
	1	SCS	3.286	2,76	1,67	-3,00	10,00
	2	SCS	2.643	2,40	1,82	-3,00	10,00
	3	SCS	2.030	2,87	1,96	-2,00	10,00
HF_90 %	1	Mkg	4.699	26,55	5,97	2,40	48,60
	2	Mkg	3.749	32,43	8,49	2,00	62,40
	3	Mkg	2.994	34,47	8,64	3,80	61,30
	1	Fkg	4.699	1,11	0,29	0,07	2,76
	2	Fkg	3.749	1,37	0,40	0,07	3,43
	3	Fkg	2.994	1,48	0,42	0,09	3,62
	1	SCS	4.685	2,61	1,73	-1,00	10,00
	2	SCS	3.737	2,71	1,94	-2,00	10,00
	3	SCS	2.984	2,99	2,02	-2,00	10,00

Das folgende gemischte lineare Modell wurde für die Prozedur Proc Mixed im Statistikprogramm SAS University Edition (SAS Institute Inc., 2008) definiert:

$$y_{ijklmn} = \mu + B_i + Lnr_j + IK(Lnr_k) + Geb_l + KS_m + A_n + e_{ijklmn,}$$

mit y_{ijklmn}= Beobachtung für lineare Milchleistungsmerkmale (Mkg, Fkg, SCS) vom ersten Testtag, μ= Mittelwert der Population, B_i = fixer Effekt für den i-ten Betrieb, Lnr_j= fixer Effekt für die j-te Laktation (1.-3.), $IK(Lnr_k)$= lineare Regression des Inzuchtkoeffizienten innerhalb Laktationsnummer, Geb_l= fixer Effekt für das l-te Geburtsjahr der Kuh (1999-2014), KS_m= fixer Effekt für die m-te Kalbesaison (Januar-März, April-Juni, Juli-September, Oktober-Dezember), A_n = zufälliger Tiereffekt für die n-te wiederholte Messung innerhalb Kuh aus verschiedenen Laktationen und e_{ijklmn}= zufälliger Restfehler.

Ergebnisse

Eigens berechnete genetische Rasseanteile in Bezug zur offiziellen Rassecodierung

Die in Tabelle 1 angegebenen genetischen Rasseanteile der als DSN sowie der als HF eingetragenen Kühe für die Kalbejahre 2005 bis 2016 und gesondert für das letzte vollständige Kalbejahr 2015 sind insbesondere in Bezug zum Verständnis der Inzuchtentwicklung eine wesentliche Grundlage. Gemäß offiziellen Beschreibungen werden für die Rassebezeichnung DSN (RC= 10) nur maximal 10 % HF-Gene toleriert (DHV, 2017). Die eigenen Berechnungen zeigen, dass insgesamt im Zeitraum von 2005 bis 2016 7.480 DSN_90 %-Kühe mit Rassecode 10 eingetragen wurden. Basierend auf den genetischen Rasseanteilen und unter Berücksichtigung der strikten Auslegung des Fremdgenanteils in der Rasse DSN gemäß offiziellen Vorgaben, wurden 3.406 Kühe (46 %) fälschlicherweise der Rasse DSN zugeordnet. Ordnet man die Tiere den Bundesländern zu, in denen sie eingetragen wurden, so wurden in den neuen Bundesländern 5 % der Kühe trotz genetischer DSN-Rasseanteile von unter 90 % als DSN mit RC= 10 eingetragen. In den alten Bundesländern konnten insgesamt 91 % falsch gekennzeichnete DSN-Tiere mit RC= 10 identifiziert werden. Im Jahr 2015 unterschritten immer noch 88 % der RC= 10 DSN-Kühe aus den alten Bundesländern und 9 % der RC= 10 DSN-Kühe aus den neuen Bundesländern die definierte Schwelle von 90 % DSN-Rasseanteil. Innerhalb der HF-Tiere in den untersuchten DSN-Betrieben wurden weniger falsch gekennzeichnete Tiere (22 %) mit HF-Rasseanteilen von unter 90 % identifiziert.

Verwandtschaften zwischen DSN und HF sowie bedeutender DSN-Bullen zur aktiven Kuhpopulation

Der durchschnittliche Verwandtschaftskoeffizient für DSN_90 % des Kalbejahrs 2015 betrug 7,6 %. Innerhalb der HF_90 % Vergleichstiere aus den „DSN-Betrieben“ konnte mit R= 6,8 % eine ähnlich hohe durchschnittliche Verwandtschaft wie für die DSN_90 % ermittelt werden. DSN_90 %-Kühe waren mit HF_90 %-Kühen mit R= 0,02 % nahezu unverwandt.

In Abbildung 3 ist die Verwandtschaft einflussreicher DSN-Bullen zur aktiven DSN-Milchkuhpopulation und DSN-Kuh-Subgruppierungen (eingeteilt nach

Milchleistungsklassen) abgetragen. Die DSN-Bullen Bedo, Nero und Best fallen hierbei durch recht hohe Verwandtschaften mit >13 % zu einzelnen Kuhgruppen auf. In der hier durchgeführten DSN-Studie zeigen sich für alle bedeutenden Bullen (Abbildung 3) höhere Verwandtschaften zu den DSN_90 %-Kühen aus der höchsten und mittleren Milchleistungsklasse.

Abbildung 4 zeigt die durchschnittlichen Verwandtschaften der DSN_90 %-Kühe innerhalb der gebildeten Milchleistungsgruppen. Die durchschnittliche Verwandtschaft der aktiven DSN_90 %-Kühe aus Herden der neuen Bundesländer war mit 9,4 % deutlich höher als von DSN_90 %-Kühen der alten Bundesländer mit nur 2,7 %.

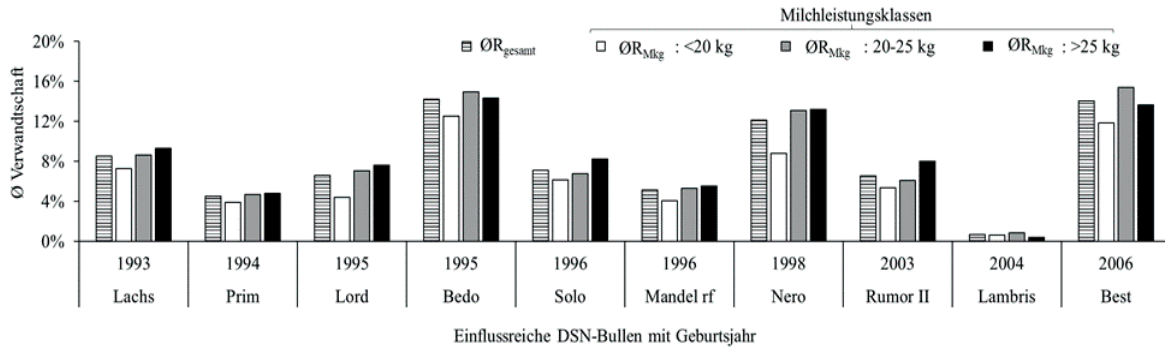

Abbildung 3: *Einflussreiche DSN-Bullen mit Geburtsjahr, deren durchschnittlicher Verwandtschaftskoeffizient zur gesamten aktiven DSN-Milchviehpopulation ($ØR_{gesamt}$), sowie deren Verwandtschaft zu DSN-Subgruppen für verschiedene Milchleistungsklassen ($ØR_{Mkg}$: <20 kg/Tag, $ØR_{Mkg}$: 20-25 kg/d, $ØR_{Mkg}$: >25 kg/Tag).*
Influential DSN sires (with birth year), their average relationship to the active DSN dairy cattle population ($ØR_{gesamt}$) as well as to the DSN dairy cows grouped into milk yielding classes ($ØR_{Mkg}$: <20 kg/d, $ØR_{Mkg}$: 20-25 kg/d, $ØR_{Mkg}$: >25 kg/d).

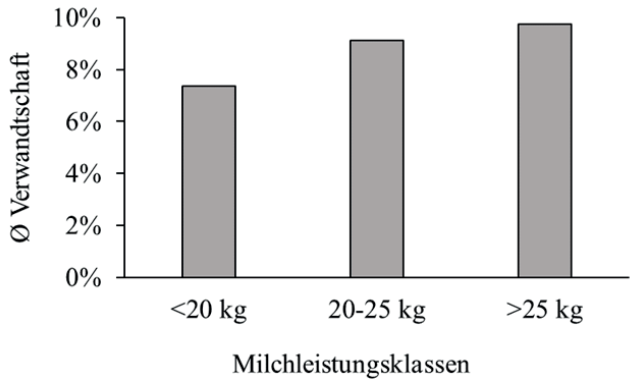

Abbildung 4: *Durchschnittlicher Verwandtschaftskoeffizient von aktiven DSN-Kühen innerhalb definierter Milchleistungsklassen.*
Average relationship coefficient of active DSN cows within groups of test-day milk yield.

Generationsintervall, Inzuchtkoeffizient und effektive Populationsgröße

Das durchschnittliche Generationsintervall auf dem Kuhmutterpfad von 1990 bis 2011 war vergleichbar für DSN_90 % (5,9 Jahre) und HF_90 % (5,7 Jahre) (Abbildung 5). Nur im Zeitraum von 1990 bis 1998 lagen die Generationsintervalle für DSN_90 % mit nahezu sieben Jahren doch deutlich über den Vergleichszahlen von HF_90 % mit 5,4 – 6,2 Jahren.

Die Inzuchtkoeffizienten für die DSN_90 %-Milchkühe und DSN-Bullen mit mehr als 20 Töchtern wurden für die Geburtsjahrgänge 1990 bis 2015 bestimmt (Abbildung 6). Für DSN-Kühe zeigt sich ein recht kontinuierlicher Anstieg der Inzuchtkoeffizienten im Jahresverlauf, während für Bullen stärkere jährliche Schwankungen auftreten. Für das Geburtsjahr 1990 betrug der durchschnittliche Inzuchtkoeffizient der DSN-Kühe 1,2 % und stieg auf 2,3 % für das Geburtsjahr 2015 an. Die effektive Populationsgröße für DSN ergab 85 DSN_90 %-Tiere in 2011 bei einem Generationsintervall von 5,9 Jahren und einer Inzuchtsteigerung von 0,1 % pro Jahr.

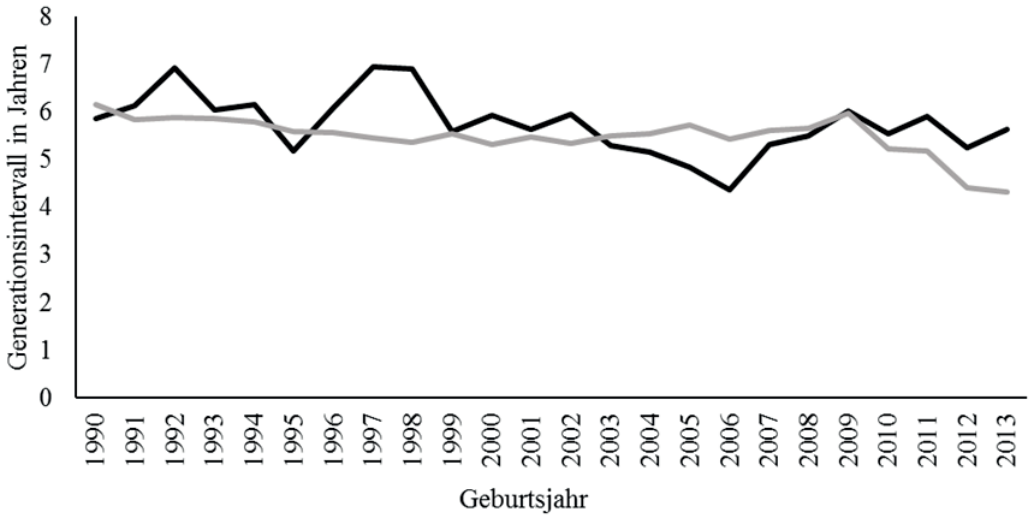

Abbildung 5: *Generationsintervalle für DSN (schwarz) und für HF (grau) für die Geburtsjahre 1990 bis 2013.*
Generation intervals for DSN (black) and for HF (grey) from birth years 1990 to 2013.

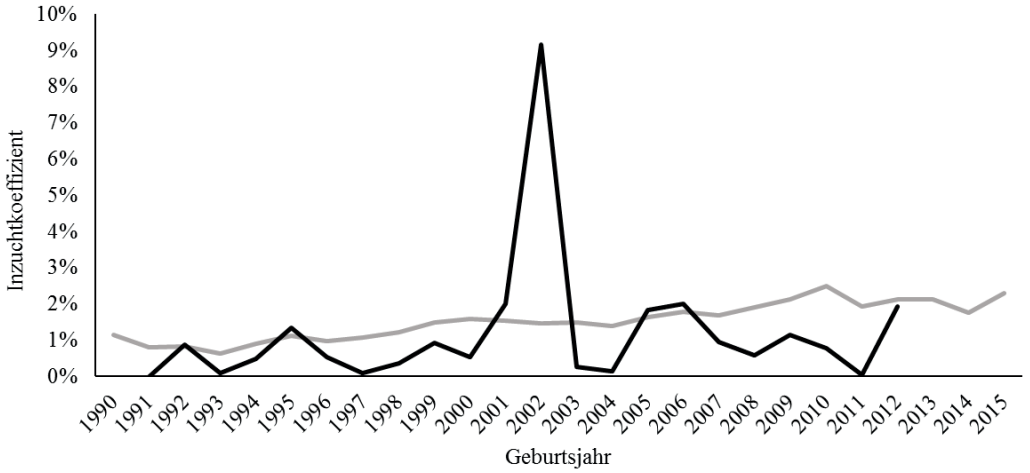

Abbildung 6: *Durchschnittlicher Inzuchtkoeffizient (F) für DSN-Kühe (grau) und Bullen (schwarz) innerhalb Geburtsjahrs von 1990 bis 2015.*
Average inbreeding coefficient (F) for DSN dairy cows (grey) and sires (black) within birth years from 1990 to 2015.

Auswirkungen von Inzucht auf Testtagsmerkmale

Mit zunehmendem Inzuchtkoeffizient konnte eine Steigerung des Merkmals Mkg in allen drei Laktationen für HF_90 % festgestellt werden (Abbildung 7). Besonders in der dritten Laktation stieg die Milchleistung um 0,58 kg pro 1 % Inzucht an. Von diesem Trend unterschieden sich die ersten beiden Laktationen der DSN_90 %, bei denen sich die tägliche Milchleistung des ersten Testtags mit zunehmendem Inzuchtkoeffizienten verringerte. In der ersten Laktation reduzierte sich die Milchleistung um 1,62 kg pro 1 % Inzuchtsteigerung. Demgegenüber stand eine geringfügige Steigerung der täglichen Milchleistung bei höheren Inzuchtkoeffizienten der DSN_90 % in der dritten Laktation.

Ein ähnliches Bild ergab sich für das Merkmal Fkg (Abbildung 8). Mit Anstieg des Inzuchtkoeffizienten erhöhte sich Fkg in der zweiten und dritten Laktation der HF_90 % und in der dritten Laktation der DSN_90 %. Analog zu Mkg verringerte sich Fkg für HF_90 % in der ersten und für DSN_90 % in der ersten und zweiten Laktation.

In der vorliegenden Studie wurden entgegen der Erwartungen keine negativen Auswirkungen von Inzucht auf SCS für DSN_90 % beobachtet (Abbildung 9). Allerdings stieg bei HF_90 % der SCS mit zunehmender Inzucht an.

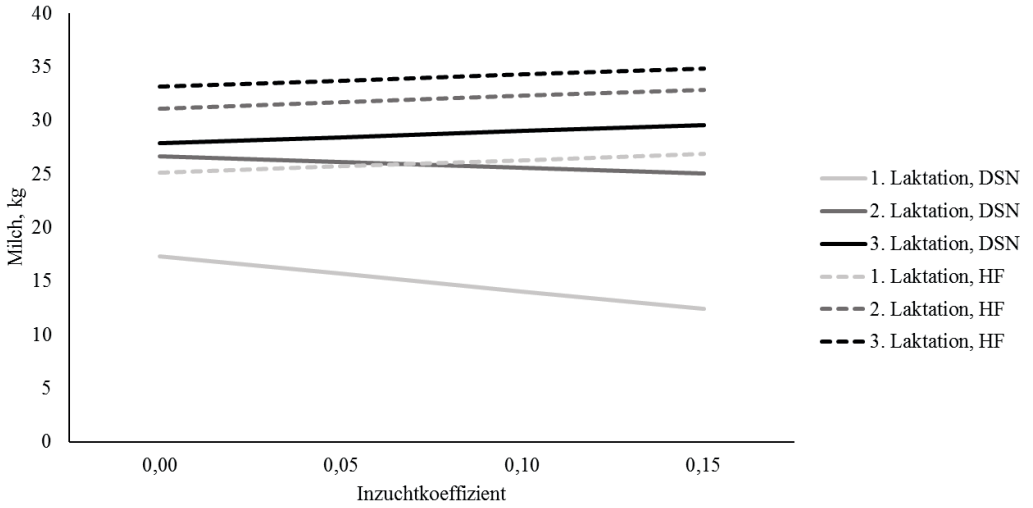

Abbildung 7: *Least Square Means für Milch-kg in Abhängigkeit der Inzuchtkoeffizienten für DSN und HF.*
Least square means of milk kg in dependency of inbreeding coefficients for DSN and HF.

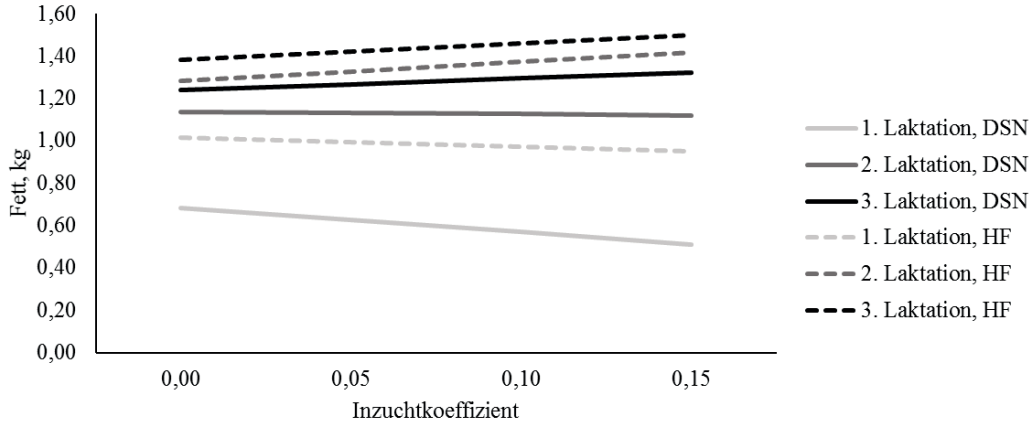

Abbildung 8: *Least Square Means für Fett-kg in Abhängigkeit der Inzuchtkoeffizienten für DSN und HF.*
Least square means of fat kg in dependency of inbreeding coefficients for DSN and HF.

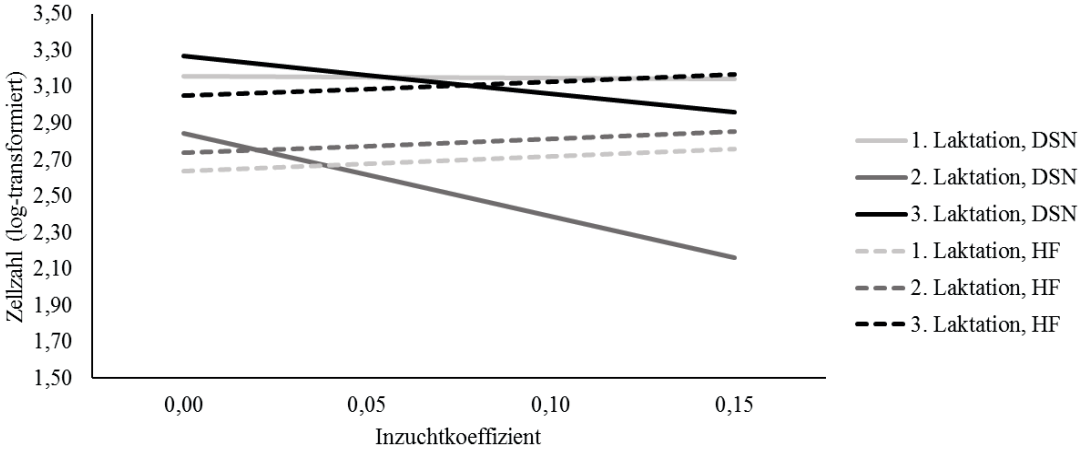

Abbildung 9: *Least Square Means für somatische Zellzahl (log-transformiert) in Abhängigkeit der Inzuchtkoeffizienten für DSN und HF.*
Least square means of somatic cell score in dependency of inbreeding coefficients for DSN and HF.

Diskussion

Rasseanteile in Bezug zur offiziellen Rassecodierung

Die Berechnungen genetischer Rasseanteile aus Tabelle 1 zeigen, dass in den Jahren von 2005 bis 2016, 46 % der DSN-Kühe und 22 % der HF-Kühe falsch gekennzeichnet wurden. Dies bedeutet, dass 3.406 Kühe den DSN und 1.703 Kühe der Rasse HF zugeordnet wurden, obwohl ihr genetischer Fremdgenanteil über 10 % lag. Selbst wenn der zulässige HF-Fremdgenanteil für DSN von 10 % auf 30 % gemäß Biedermann et al. (2005) hochgesetzt würde, wären noch immer 52 % der Tiere aus den alten Bundesländern, aber 0 % der Tiere aus den neuen Bundesländern, falsch gekennzeichnet. Mögliche Gründe für die unterschiedlich strikten Rassekennzeichnungen zwischen alten und neuen Bundesländern sind wohl die zentrale Organisation der DSN-Zucht durch den RBB und die Konzentration des DSN-Hauptbestands auf nur zwei Großbetriebe, wobei in den alten Bundesländern keine vergleichbaren intensiv organisierten Zuchtstrukturen und Zuchtprogramme für DSN vorliegen. Zudem werden die Züchter in den alten Bundesländern dazu ermutigt, Anpaarungen von DSN-Kühen mit HF-Bullen durchzuführen, da Nachkommen aus diesen „Kreuzungen“ als deutsche Holsteins im Herdbuch eingetragen werden dürfen (DHV, 2017). Biedermann et al. (2005) recherchierten nach den Ursachen für variierende genetische Rasseanteile in den DSN. Dabei identifizierten sie Züchter, die nur über einen sehr kurzen Zeitraum HF-Bullen einsetzen, oder solche, die über eine Verdrängungszucht erneut Herden des alten Typs aufbauen wollen.

Verwandtschaften

Der ermittelte Verwandtschaftskoeffizient der DSN_90 % (Kalbejahr 2015) (7,6 %) liegt sehr viel höher, als der damals von Biedermann et al. (2005) ermittelte Wert (4,6 %) für 423 aktive DSN-Kühe und 140 aktive DSN-Bullen. Eine Ursache für die höhere Verwandtschaft in der aktuellen DSN-Studie ist wohl darauf zurückzuführen, dass Biedermann et al. (2005) 30 % Fremdgenanteil zu ließen.

Auch in Bezug zu anderen bedrohten Rassen wie dem Vorderwälder Rind mit R= 3,5 % (Biedermann et al., 2004) sind die DSN untereinander enger miteinander verwandt. Allerdings muss bei derartigen Vergleichen immer bedacht werden, dass die Tiefe der

berücksichtigten Abstammungsdateien in verschiedenen Studien variierte und somit der Inzuchtzuwachs eine bessere Maßzahl für neutrale Populations- oder Rassevergleiche wäre. In einer intensiv vorselektierten kleineren Subpopulation von HF-Bullenmüttern und HF-Bullenvätern mit ähnlicher Populationsgröße, analog zur DSN-Population, fanden König und Simianer (2006) mit R= 5,9 % ähnliche Verwandtschaften wie in der aktuellen DSN-Population. Aber auch innerhalb von großen Holsteinpopulationen unter Berücksichtigung kommerzieller Produktionskühe wurden schon vor ca. 20 Jahren vergleichbare Verwandtschaftskoeffizienten berechnet (u.a. R= 10,2 % in der amerikanischen Holsteinpopulation (Young und Seykora, 1996). Im genomischen Zeitalter ist innerhalb HF ein weiterer deutlicher Anstieg der Verwandtschaftskoeffizienten zu beobachten (König et al., 2018).

Auffallend hohe Verwandtschaften (R> 13 %) der DSN-Bullen Bedo, Nero und Best zu einzelnen Kuhgruppen können zum einen durch deren große Töchterzahlen bedingt sein, zum anderen aber auch durch den starken Einsatz ihrer eigenen Väter und Söhne über künstliche Besamung erklärt werden. So ist Bedo der Vater zweier einflussreicher DSN-Bullen wie Berko und Best. Darüber hinaus hat Nero mit Nestor und Nimbus zwei bedeutende Söhne. Nero prägte als Muttersvater auch die Linie von Larus und Sony. Bereits Biedermann et al. (2005) hoben die Bedeutung der Bullen Solist und Renegat hervor, für welche durchschnittliche Verwandtschaften zur DSN-Kuhpopulation von 2,8 % und 4,7 % berechnet wurden. Da auch Semper, Hermes und Nimbus (nicht abgebildet) von einflussreichen Vätern wie Solo, Hanno und Nero abstammen, waren höhere Verwandtschaftskoeffizienten für diese Bullen bzw. Blutlinien zu erwarten. Auch bewusst herbeigeführte Inzucht im Falle sehr leistungsfähiger Vaterlinien zur Konsolidierung besonders wünschenswerter Anlagen ist eine Begründung für höhere Verwandtschaften bestimmter Bullen zur Kuhpopulation. Dies trifft beispielsweise für den DSN-Vererber Renegat zu. Renegats Vater ist Reaktor (Geburtsjahr 1979), dessen Vater wiederum Remus ist. Mütterlicherseits ist Remus ebenfalls als Muttersvater in Renegats Vorfahren enthalten.

König und Simianer (2006) berechneten Verwandtschaften zwischen einflussreichen HF-Bullen zu Kuhgruppen mit hohen oder niedrigen Zuchtwerten für verschiedene Relativzuchtwerte. Somit konnten in der HF-Studie Rückschlüsse gezogen werden, welche Vererber oder Blutlinien zur Verbesserung, aber auch Verschlechterung, einzelner Merkmalskomplexe beigetragen haben. Es wurden vergleichsweise hohe

Verwandtschaftskoeffizienten bedeutender Bullen zu DSN-Kühen aus der höchsten und mittleren Milchleistungsklasse berechnet, wohingegen die Verwandtschaftskoeffizienten zwischen diesen Bullen und den DSN_90 %-Kühen der niedrigsten Milchleistungsklasse geringer waren. Der Grund für diese geringeren Verwandtschaften ist eine weniger starke Selektion auf Milchleistung, aber womöglich auch der verstärkte Einsatz von ungeprüften Deckbullen.

Die durchschnittlichen Verwandtschaften innerhalb Leistungsgruppen reflektieren auch auf der Ebene der Kuhpopulation den Selektionseffekt bzw. den Fokus auf bestimmte Bullen und Bullenmütter (Abbildung 4). Mit zunehmendem Leistungsniveau wurden höhere Verwandtschaften innerhalb der Tiergruppen festgestellt. Insbesondere der Verwandtschaftskoeffizient von fast 10 % in der Kuhgruppe mit dem höchsten Milchleistungsniveau ist durch die intensive Nutzung von wenigen sehr guten Leistungsvererbern begründet. Pirchner et al. (2002) argumentierten ähnlich und nannten eine verstärkte Selektion auf Milchleistung als Grund für höhere Inzuchtraten beim Tiroler Grauvieh, was dementsprechend eine höhere mittlere Verwandtschaft widerspiegelte.

Deutliche Unterschiede zwischen Verwandtschaften der aktiven DSN_90 %-Kühe aus Herden der neuen Bundesländer verglichen mit aktiven DSN_90 %-Kühen der alten Bundesländer lassen sich auf die Nutzung künstlicher Besamung, besonders in Ostdeutschland, zurückführen. Insbesondere strikte Reinzucht und Minimierung des Fremdgenanteils innerhalb der Ostbetriebe könnten Begründungen für diese vergleichsweise hohe Verwandtschaft sein.

Generationsintervall, Inzuchtkoeffizient und effektive Populationsgröße

Die Generationsintervalle der DSN_90 % (5,9 Jahre) und HF_90 % (5,7 Jahre) auf dem Kuhmutterpfad sind vergleichbar mit Werten für andere vom Aussterben bedrohte Rassen wie dem Tiroler Grauvieh (5,7 %), aber auch größeren Populationen wie den Dänischen Holsteins (4,6 %-5,0 %), den Dänischen Jerseys (4,7 %-5,2 %) und dem Dänischen Rotvieh (4,8 % - 5,0 %) (Mészáros et al., 2015; Sørensen et al., 2005). Für kleine sowie große Milchkuhpopulationen gelten somit auf dem Kuhmutterpfad der Selektion ähnliche Selektionsstrategien und es ist auch fraglich, ob das Werkzeug der genomischen Selektion diesbezüglich innerbetriebliche Veränderungen bewirken wird (König et al., 2018). Ein überraschend geringes Generationsintervall von nur 4,9 Jahren ermittelten Ehling et al.

(1999) für den DSN-Bestand in Mariensee. Im Rahmen eines Erhaltungszuchtprogramms werden eher lange Generationsintervalle angestrebt, um den durchschnittlichen Inzuchtzuwachs pro Jahr zu minimieren (Kehr et al., 2009).

Der kontinuierliche Anstieg der Inzuchtkoeffizienten im Jahresverlauf der DSN-Kühe unterscheidet sich gravierend vom Inzuchtverlauf der DSN-Bullen. So ist der Peak im Inzuchtkoeffizient für Bullen für das Geburtsjahr 2002 auf den starken Einsatz des DSN-Vererbers Jochem zurückzuführen. Der hohe Inzuchtwert des Bullen Jochem (25,8 %), welcher sich durch seine ingezüchteten Vorfahren begründet, stellt nur ein Beispiel des von Biedermann et al. (2004) beschriebenen Flaschenhalseffektes auf paternaler Seite dar. Weitere Bullen, die substanziell zur Inzuchtentwicklung innerhalb der DSN beigetragen haben, waren Semper (1,7 %), Hermes (2,5 %) und Nimbus (3,4 %). Neben Jochem fiel auch Renegat mit einem recht hohen Inzuchtkoeffizienten von 5 % auf. Auch VanRaden und Smith (1999) berechneten Inzuchtkoeffizienten für einzelne HF-Bullen, mit Maximalwerten von 7,9 % für To-Mar Blackstar und 7,7 % für R.O.R.A. Elevation.

Moderate Steigerungen der durchschnittlichen Inzuchtkoeffizienten der DSN-Kühe der Geburtsjahre 1990 (1,2 %) bis 2015 (2,3 %) sind vergleichbar mit Werten für DSN aus älteren Studien. So schätzten Ehling et al. (1999) für die DSN-Kühe in Mariensee einen Inzuchtkoeffizienten von 1,27 % in 1995, welcher mit dem Inzuchtkoeffizienten aller DSN_90 %-Kühe des Geburtsjahres 1995 (1,1 %) in Einklang ist. Studien für amerikanische Holsteins zeigten einen vergleichbaren Inzuchtkoeffizienten mit dem der DSN-Kühe für das Geburtsjahr 1999, aber wiesen ein sehr viel höheres Inzuchtniveau für 2015 auf (CDCB, 2017). Dies könnten schon Auswirkungen der genomischen Selektion, mit einer Fokussierung auf weitestgehend identische HF-Genetik weltweit, sein.

Basierend auf der Inzuchtentwicklung fiel die effektive Populationsgröße der DSN_90 % in 2011 mit 85 Tieren gering aus. König und Simianer (2006) berechneten in 1999 eine effektive Populationsgröße von 52 Tieren basierend auf der Inzuchtentwicklung deutscher Holstein-Kühe. Medugorac et al. (2009) bewerteten die effektive Populationsgröße von 142 Tieren bei Murnau-Werdenfelsern als kritisch, und identifizierten als Gründe den Einsatz von nur drei Vaterlinien, umfassende künstliche Besamung und wenig diversifizierte Zuchtprogramme. Hartwig und Bennewitz (2014) hingegen beurteilten eine effektive Populationsgröße zwischen 50 und 100 Tieren (basierend auf der Inzuchtentwicklung) als ausreichend genetisch divers. Laut der

Bundesanstalt für Landwirtschaft und Ernährung (TGRDEU, 2018) ist eine Population mit N_e< 200 Tieren (N_e-Berechnung basierend auf der Anzahl männlicher und weiblicher Tiere) als stark existenzgefährdet einzustufen. Aktuelle Bestandszahlen der TGRDEU aus 2016 verzeichneten 71 DSN-Bullen und 2.847 DSN-Kühe (N_e= 277, basierend auf der Anzahl männlicher und weiblicher Tiere). Es muss an dieser Stelle erwähnt werden, dass effektive Populationskennzahlen, welche auf verschiedener Rechenmethodik basieren (Inzuchtanstieg oder Geschlechterverhältnis), nicht unmittelbar miteinander vergleichbar sind.

Auswirkungen von Inzucht auf Testtagsmerkmale

Heterogene Ergebnisse für den Einfluss der Inzuchtkoeffizienten auf die Produktionsmerkmale Mkg und Fkg in den Rassen DSN_90 % und HF_90 % bedürfen weiterer Studien. Jedoch fanden auch Smith et al. (1998) unterschiedliche Inzuchtauswirkungen für verschiedene Produktions- und Exterieurmerkmale. Negative Auswirkungen von Inzucht auf Mkg und Fkg in den ersten beiden Laktationen der DSN_90 % sind in Einklang mit Ergebnissen von Croquet et al. (2006) für belgische Holsteins. Am Beispiel amerikanischer Jerseys, die in etwa das Milchleistungsniveau der DSN reflektieren, zeigten Thompson et al. (2000) und Cassell et al. (2002) ebenfalls negative Inzuchteinflüsse auf Milchleistungsmerkmale.

Für das funktionale und niedrig erbliche Merkmal SCS fanden Thompson et al. (2000) jedoch keinerlei Inzuchtdepressionen, was in der vorliegenden Studie durch die Ergebnisse der DSN_90 % bestätigt wurde. Allerdings wurde ein Anstieg des SCS mit zunehmender Inzucht bei HF_90 % beobachtet. Auch Miglior et al. (1995) berichteten von erhöhten SCS von HF-Kühen mit hohen Inzuchtkoeffizienten. Sørensen et al. (2006) begründeten höhere SCS-Werte durch geringere Mastitis-Resistenzen von stärker ingezüchteten HF-Kühen. Die unterschiedlichen Auswirkungen von Inzucht beim Vergleich HF_90 % mit DSN_90 % kann insbesondere beim Merkmal Zellzahl durch unterschiedliche Selektionsstrategien begründet sein. Innerhalb der DSN-Rasse gibt es nur wenige Vererber für hohe Zellzahlzuchtwerte, die gleichzeitig umfangreich eingesetzt wurden. Insgesamt konnten nur wenig DSN_90 %-Kühe mit hohem Inzuchtniveau (≥ 10 %) identifiziert werden. Bei niedrigem Ausgangsniveau der Inzucht sind auch generell weniger Inzuchtdepressionen zu erwarten. Dies ist vor dem Hintergrund zukünftiger

Inzuchtentwicklungen und der Weiterentwicklung züchterischer Strategien für DSN als überausaus positiv zu bewerten.

Schlussfolgerung

Eigene Berechnungen zu genetischen Rasseanteilen der DSN und HF zeigten, dass überwiegend DSN-Tiere (Rassecode= 10) aus den alten Bundesländern über mehr als den erlaubten HF-Fremdgenanteil von 10 % verfügten. So wurden in den Kalbejahren von 2005 bis 2016 insgesamt 46 % der Kühe fälschlicherweise der DSN-Rasse zugewiesen. Dieses Ergebnis lässt deutliche Rückschlüsse auf unterschiedliche Zuchtstrategien in Ost- und Westdeutschland zu, mit nur wenigen „reinen“ DSN-Zuchtbetrieben im Westen. Hier überwogen Anpaarungen von HF-Bullen mit DSN-Kühen, wohingegen die untersuchten Betriebe in den neuen Bundesländern strikte DSN-Reinzucht verfolgten. Bezüglich der Verwandtschaftsstrukturen zeigten insbesondere höher leistende DSN-Milchkühe engere Verwandtschaften zu bedeutenden DSN-Bullenlinien. Dies legt den Schluss nahe, dass geprüfte und bekannte Milchmengenvererber über künstliche Besamung intensiv genutzt wurden und niedrig leistende Herden doch verstärkt Natursprungbullen genutzt haben.
Obwohl die Inzuchtsteigerung für DSN_90 % von 2006 bis 2011 bei lediglich 0,1 % pro Jahr lag, sollte sie zukünftig kritisch beobachtet werden. Eine aus dieser Inzuchtsteigerung resultierende effektive Populationsgröße von nur 85 Tieren unterstreicht die Notwendigkeit eines Monitorings der Rasse DSN oder weiterführend den Einsatz von Anpaarungsprogrammen unter Berücksichtigung der Entwicklung von Inzucht und Verwandtschaft. Für Milchleistungsmerkmale in der Frühlaktation konnten keine ausgeprägten Inzuchtdepressionen, weder für DSN_90 % noch für HF_90 %, nachgewiesen werden. Allgemein gingen höhere Inzuchtkoeffizienten mit einer Steigerung der Milch- und Fettleistung in allen drei Laktationen für HF_90 % einher. Von diesem Trend unterschieden sich die ersten beiden Laktationen der DSN_90 %, wo sich die tägliche Milchleistung des ersten Testtags mit zunehmenden Inzuchtkoeffizienten reduzierte. Demgegenüber stand ein geringfügiger Anstieg der täglichen Milchleistung bei höheren Inzuchtkoeffizienten der DSN_90 % in der dritten Laktation. Steigende Inzuchtkoeffizienten führten sogar zu verringerten somatischen Zellzahlen für DSN_90 % in der zweiten und dritten Laktation. Diese Ergebnisse widersprachen den Entwicklungen der HF_90 %, wo mit steigender Inzucht erhöhte Zellzahlen in der zweiten und dritten Laktation beobachtet wurden. An dieser Stelle bedarf es

weiterführender vertiefender Analysen, um die unterschiedlichen Reaktionen zwischen HF und DSN auf Inzucht genau erklären zu können. Es sind doch deutlich verschiedene Rassen, da der Verwandtschaftskoeffizient zwischen HF und DSN nur 0,02 % beträgt.

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CHAPTER 3

Associations between Precision Sensor Data with Subjectively Scored Cattle Welfare Indicators, Test-Day Production, Health and Fertility Traits in Native Black and White Dual-Purpose Cattle Kept in Pasture-Based Production Systems

Introduction

With the abolition of the European Union milk quota system in April 2015 rose the opportunity for dairy farms to expand, causing substantial structural changes in the dairy sector (Klootwijk et al., 2016). Together with co-drivers such as technical change and altering macroeconomic conditions, a steady herd-size evolution led to increasing dairy cow numbers per herd (Huettel and Jongeneel, 2011). In Germany 2013, the average herd size comprised 54 cows, but increased up to 64 cows per herd in 2017 (©DIALOG-MILCH, 2018). As labour expenses are one major contributor to milk production costs in industrialized countries, mechanization and precision management have been progressively implemented, changing the daily work routine of farmers. However, due to mass handling of large dairy cattle groups, only limited time is available for individual animal welfare supervision.

From such a perspective, an alternative solution for subjective dairy cattle observation is the establishment of precision dairy monitoring and managing techniques. Nowadays, automatically assessing feeding, ruminating, activity, heat and calving behaviour, as well as ruminal pH, reticular contraction, acoustics, progesterone, vaginal mucus electrical resistance, surface temperature, respiration rate, heart rate measurement is possible. Next to animal welfare aspects, dairy farmers have to meet extensive consumer demands, which can be fulfilled by the usage of precision dairy farming (PDF): Continuous quality control, natural pathogen-free food, prevention of zoonotic disease transmission and minimizing medical treatment (Bewley et al., 2010). Additionally, data generated by PDF can be used for future genetic evaluations for functional traits, in order to improve animal health and thus longevity (Bewley et al., 2010). With increasing incidences of health disorders and mortality rates from 2 to 3.5 % within the last 10 years, early identification of distressed animals can improve animal welfare and helps to minimize costs (Thomsen et al., 2004). In many cases, it is too late and very costly to take actions, by the time the animal exhibits clinical signs of distress, as obvious clinical symptoms are usually the consequence of preceding physiological disruptions, that are difficult to detect (e.g. change in temperature, heart rate). Hence, with the recording of on-farm real time behaviour/ health data, physiological changes may be noticed right at the beginning, enabling an early intervention and thus, improving treatment efficiency (Bewley et al., 2010).

Ample evidence from experimental studies emphasizes the importance of modern farm technology implementation. Quimby et al. (2001) showed, that measuring feeding time at the feed bunk via radiofrequency technology contributed to the detection of morbid beef cattle 4 days earlier than only visually monitoring animals by experienced farm staff. Additionally, Dutta et al. (2015) described the possibilities of machine-learning techniques, as collar systems (3-axis accelerometer) and magnetometers, accurately infer and classify physical cow behaviour (ruminating, grazing, walking, searching, chewing scratching/ grooming).

Especially daily behaviour such as rumination, eating and activity patterns are commonly respected indicators, relating well to animal health (Bikker et al., 2014). Thus, automated behaviour observation may provide a useful tool to monitor cattle, especially before the time of calving, as various behavioural parameters (e.g. eating, ruminating) change within the days prior to parturition (Rutten, 2017). The electronic behaviour 'CowManager SensOor system' (Agis Automatisering BV, Harmelen, the Netherlands) promises real-time quantification of (multi-point) ear temperature, ruminating and feeding behaviour, as well as activity of milking cows. A literature overview, addressing validations of the CowManager SensOor system, is given in Table 3. Sensors may be capable to detect gradual changes in feeding, ruminating and activity, and they give early 'health' alerts. Up to this point, various studies have been conducted on the subject of PDF in Holstein Friesian (HF) herds. However, other cattle breeds, especially dual-purpose cattle, vary in dry matter intake (DMI), milk production and composition, live weight, body conformation and breed typical behaviour patterns from HF (Palladino et al., 2010). Consequently, resilient dual-purpose cattle might react differently on environmental stimuli. Typical breed behaviour patterns interacting with environmental influences suggest a broad validation of sensor technology, i.e. additionally relating sensor data to novel health and welfare indicator traits under grazing conditions.

To our knowledge, behaviour data recorded by sensor, has not yet been statistically evaluated and put into relationship with conventional production or welfare assessment traits, especially against the background of dual-purpose cattle kept in alternative outdoor or grazing systems. Hence, the present study aims at identifying associations between longitudinal sensor data recording for feeding, rumination, activity and sleeping/lying behaviour as well as ear temperature with own subjective scores for welfare indicator

traits. As a further challenge, novel trait recording under grazing conditions in local black and white dual-purpose cattle (DSN) was conducted. Correlations between sensor traits with productivity and novel welfare assessment traits are imperative when i) enhancing DSN breeding goals via longitudinal sensor data, and ii) aiming on the optimisation of preventive DSN health management in grazing systems.

Table 3: Literature overview of CowManager (CM) SensOor studies with regard to tested traits: feeding, rumination, active, high active, lying/sleeping, ear temperature.

Reference	Aim of Study, sensor traits	Method	Country	No. animals	Findings
Bikker et al. (2014)	Validation of CM recordings via visual observation (ruminating, active, lying)	-Pearson correlation - CCC	Netherlands, Feb.-Mar.2013 1 Farm Freestall barn	15 HF cows	(−) Eating: percentage recorded by CM 2.9 % higher than visual observation, minute-by-minute agreement (κ=0.77) (+) Ruminating: minute-by-minute agreement was almost perfect (κ value=0.85) (/) Active: percentage recorded by CM 5.0 % lower than visual observation, minute-by-minute agreement (κ=0.47) (+) Lying: percentage recorded by CM 1.6 % units higher than visual observation, minute-minute agreement (κ=0.86) ➢ Significant differences between visual observation and CM → CM recordings are likely to be more accurate ➢ Ear movement relating to eating and active seem too complex, are more difficult to classify than rumination ➢ Research design not suited for oestrus detection
Borchers et al. (2016)	Validation of CM recordings via visual observation (eating, ruminating)	-Pearson corr. - CCC	US, Kentucky Freestall barn	48 HF cows	(+) Eating: visual behaviour observation correlated well with CM (r= 0.88, CCC=0.82) (−) Ruminating: visual observation correlated weakly with CM (r= 0.69, CCC=0.59)
Dolecheck et al. (2015)	1) Detection of oestrus-relate changes (eating, ruminating, active, ear temp.) 2) Exploration of oestrus detection potential of machine-learning techniques	-Random forest, -Linear discr. analysis, -Neural network	US, Kentucky Freestall barn	32 HF cows	(+) Eating, Ruminating, Active min./h: Identification between oestrus and non-oestrus possible (−) Ear surface temperature: No oestrus identification ➢ Machine-learning techniques have potential to be applied to automatically collected technology data for oestrus detection
Ouellet et al. (2016)	CM performance testing of calving onset prediction (ruminating, lying)	-Pearson correlation, -Glimmix, -ROC /AUC	Canada, Nov. 2013-Jun. 2014	42 HF cows	(+) Ruminating: mean decrease of 41±17 min/24 h ($P < 0.05$) on calving day compared 4 days pre-calving. Time of the day had a significant effect ($P<0.01$) on rumination time (+) Lying: reduced on calving day compared to 4 days pre-calving (amplitude: mean ± SE; 52 ± 28 min). Time of day had effect ($P<0.001$) on lying time (increased during night, reduced in afternoon) ➢ Lower calving prediction performance of rumination and (lower sensitivity, specificity) than vaginal temperature: "Physiological changes might be more accurate for calving time prediction, than behavioural ones"
Rutten et al. (2015)	CM performance testing of calving onset prediction (ruminating, active, high active, lying, ear temperature)	-Logit models -ROC/ AUC	Netherlands, 2 Farms 1 year	450 cows	➢ Accurate prediction of calving start ➢ High number of false positive and false negative alarms
Rutten (2017)	Calving onset prediction (eating, ruminating, active, high active, lying, ear temperature) 1) At which moment does CM give reliable calving prediction value 2) Prediction accuracy of calving onset 3) Model validation of calving onset prediction	-Logit models -ROC /AUC	Netherlands, Sept. 2013-Nov. 2014	400 cows	(−) Eating: no change before calving. (+) Ruminating: decrease (15min/h) in the hour of calving. (+) Active: contributed the most to the model, regarding AICc. (−) High active: contributed least to the model, regarding AICc. (−) Lying: no change before calving. (+) Temperature: second highest impact on model, regarding AICc. Changes in CM visible 12 h before calving but specific alert for the onset of calving not feasible ➢ ROC for expected calving date as independent variable: 0.885 (sensitivity: 9.1 %) ➢ ROC for independent variables based on CM data: 0.929 (sensitivity: 36.4 %) ➢ Combining CM parameters led to lowest AICc
Wolfger et al. (2015)	Sensitivity and specificity accuracy of CM to detect rumination and feeding behaviour in beef cattle (eating, ruminating, active, lying)	-Mixed model logistic regression -CCC	Canada, August: 13 days during day time	18 yearling steers Hereford ×Angus	(+) Eating: sensitivity 95 %, specificity: 76 %; CCC: 0.79 (95 % CI: 0.61 – 0.85) (/) Ruminating: sensitivity 49 %, specificity: 96 %; CCC: 0.44 (95 % CI: 0.23 – 0.60) ➢ Variability among steers ranged for ruminating (CCC 0.09 to 0.98) and feeding (0.58 to .096) ➢ "Promising monitoring system for feeding behaviour" ➢ Associated algorithm needs to be optimized to better differentiate rumination from feeding

AUC=Area under the curve; AICc=Akaike's Information Criterion; CCC=Concordance correlation coefficient; ROC=Receiving operating characteristics; κ= κ-value; (+)=positive findings (well-functioning) of CM, (−)=negative findings (poor functioning) of CM; (/)=intermediate functionality of CM

Materials and Methods

Welfare assessment and temperament trait recording

The study included 118 DSN cows from parities one to four, kept on an organic research farm in Hessen, Germany. The grazing season ranged from the beginning of April until the end of November, with a daily pasture access of 6 hours. A weather station, belonging to the research farm, recorded daily temperature and humidity on an hourly basis. Subjective recording of welfare assessment traits (WAT) included body condition score (BCS: scale 1= emaciated to 5= obese) (Spengler Neff et al., 2015), locomotion score (LS: scale 1= normal to 5= severely lame) (Zinpro Corporation, 2018), and hygiene scores for the udder (UHS: scale 1= clean to 5= manure encrusted) and for legs (LHS: scale 1= clean to 5= manure encrusted) (Reneau et al., 2005). The same trained observer recorded all traits five times, beginning in April 2016 until May 2017. In total, 451 observations were available for data processing. A trained person recorded temperament traits according to Juga (1996) of 67 DSN cows twice (July 2016 and May 2017). In this regard, general temperament (GT) during milking (scale: 1= nervous to 5= calm), aggressiveness (AGG) towards herd mates (scale: 1= aggressive; 0= untroubled) and intra herd rank order (IHRO) (scale: 1= submissive; 2= medium rank; 3= dominant) were recorded. In the following, GT, AGG and IHRO are defined as temperament traits.

Sensor trait recording

The automated CowManager technology (by Agis Automatisering BV) was chosen due to its practicability and broad pattern of longitudinal trait recording (Bikker et al., 2002). Previous sensor studies stated the device's high value and contribution to research (Table 3). In order to generate a longitudinal data structure of electronically recorded behaviour traits, 49 DSN cows were equipped with sensors. The sensor was implemented at least 2 days prior to the expected calving date. Once implemented into the cow's left ear, the sensor system uses a 3-dimentional accelerometer (attached to radio frequency identification tag) to match records of every minute to five behaviour categories (rumination= RUM, feeding= FEED, sleeping/lying/ not active= NA, moderate activity= ACT, high activity= HA). Besides behaviour recordings, the sensor uses a digital surface temperature monitor to measure the mean hourly ear surface temperature (ET). While the

system detects RUM based on the typical repetitive ear movements caused by the animal's chewing and regurgitation, FEED relates to the cow's food intake expressed through masticatory movement. The activity parameters are subcategorized into basic ACT, HA and no activity NA. The state of ACT describes any kind of moderate ear movements resulting from walking, head shaking etc., that cannot be associated with the repetitive ear movements during rumination or feeding. High activity implies increased ACT, which in most cases is elevated prior to parturition or during oestrus, including mounting behaviour. No activity refers to a minimum or no ear movement while the cow sleeps or rests (usually when lying down). The hourly percentage for all sensor traits is transmitted through a wireless connection to a router within the stable. Consequently, whenever the sensor records a certain behaviour such as RUM, it does not assign this time to another behaviour trait (e.g. NA), implying collinearity for the sensor traits.

Data processing and statistical analysis

Outlier removal for sensor traits included daily mean values that were smaller than 5 % or larger than 90 %, applying the UNIVARIATE procedure of JMP (SAS Institute Inc., 2008). For ET, reasonable records ranged between > 5 °C and < 40 °C. Daily temperature and humidity records from the on-farm weather station were merged with daily sensor records. In a second step, the daily mean of each sensor trait was merged with test-day traits for milk yield (Mkg), fat yield (Fkg), somatic cell score (SCS) and somatic cell count (SCC), and with the WAT from the same date. Pearson correlation coefficients were calculated among sensor traits, and between WAT and temperament traits, applying the CORR procedure of SAS. Furthermore, mixed model analysis (as implemented in the SAS MIXED procedure) was applied to infer associations between sensor, test-day traits and WAT.

The first mixed model (1) was defined to analyse the influence of sensor traits on production traits (i.e., the sensor trait was the explanatory categorical fixed effect). Model (1) was chosen, because our intention was to test if sensor trait behaviour thresholds can explain variation in cow productivity.

1) $y_{ijklmn} = \mu + CS_i + Lno_j + DIM_k + A_l + ET*Temp_m + S_n + e_{ijklmn}$

The second model (2) was a recursive model in analogy to model (1), i.e., analysing the influence of production traits on sensor traits (i.e., the production trait category was the explanatory categorical fixed effect). The reason behind this modelling was to get deeper insight into behaviour patterns, depending on production levels. Norring et al. (2012) identified a strong impact of milk yield on lying and feeding behaviour in Ayrshire cows. Hence, model (2) was defined to study those associations in dual-purpose cows under grazing conditions. Model (2) was:

2) $y_{ijklm} = \mu + Lno_i + DIM_j + A_k + ET*Temp_l + P_m + e_{ijklm}$

In the third model (3), the influence of sensor traits on WAT (i.e., the sensor trait was the explanatory categorical fixed effect) was investigated. It was hypothesised that certain sensor behaviours may directly be linked to the body condition, hygiene or locomotion of cows. Knowledge of such associations is imperative when using sensor traits as possible indicators for energy efficiency (e.g. BCS), health (e.g. locomotion) or welfare traits (e.g. hygiene scores) in selection index calculations when defining overall breeding goals. Model (3) was:

3) $y_{ijkl} = \mu + Lno_i + DIM_j + A_k + S_l + e_{ijkl}$

where, y_{ijklmn} was the observation for production traits (Mkg, Fkg) of test-day records; y_{ijklm} was the observation for sensor traits (RUM, FEED, ACT, HA, NA, ET); y_{ijkl} was the observation for WAT (LS, BCS, UHS, LHS); CS_i was the fixed effect for calving season (April-November, December-March); Lno_j and Lno_i were the fixed effects for parity classes (1-4), DIM_j and DIM_k were the fixed effects of days in milk classes (9 classes: 1-50 d, 50-100 d, until 250-300 d, 300-400 d, 400-500 d, >500 d); DIM_j in model (3) was the fixed effect of days in milk at the WAT recording date (3 classes: 0-50 d, 50-150 d, >150 d); A_l and A_k were random cow effects; $ET*Temp_m$ and $ET*Temp_l$ were combined fixed effects of ET and the outside temperature; S_n and S_l were the fixed effects for the daily mean of sensor traits (NA in %/d: <22, 22-28, >28 or FEED in %/d: <17, 17-23, >23 or RUM in %/d: <37, 37-41, >41; ACT in %/d: <6, 6-8, >8; HA in %/d: <6, 6-8, >8); P_m was the fixed effect of production trait classes (Mkg: <15, 15-17, 17-20, 20-25, 25-30, >30, or SCC*1000: <50 , 50-100, 100-150, 150-200, 200-250, 250-300, 300-350, 350-450, 450-550, 550-700, >700); e_{ijklmn}, e_{ijklm} and e_{ijkl} were the random residual effects. When modelling the impact of production traits on sensor traits, of sensor traits

on production traits, or of sensor traits on WAT, the different explanatory variables were tested in consecutive separate runs.

Results

Correlations among sensor traits and between WAT and temperament traits

Aside from the negative correlation between ET with FEED (-0.17; $p< 0.01$) and between ET with NA (-0.21; $p< 0.001$), positive relations were observed between ET with RUM (0.10; ns), ET with HA (0.31; $p< 0.001$) and ET with ACT (0.20; $p<0$ .001). Further correlation coefficients among sensor traits reflect the data's collinearity. Hence, whenever the cow spends time with FEED, all other traits (RUM, ACT, NA, HA) decrease, leading to negative correlations. The remaining sensor trait correlation coefficients were: RUM and FEED: -0.14, RUM and HA: -0.25, RUM and NA: -0.43, RUM and ACT: -0.23, FEED and HA: -0.34, FEED and NA: -0.41, FEED and ACT: -0.36, HA and NA: -0.17, HA and ACT: 0.32, NA and ACT: -0.29.

Correlations between subjectively scored WAT and temperament traits are depicted in Table 4. Correlations were also positive and highly significant between both hygiene scores UHS and LHS (0.65; $p< 0.001$). Correlations were significantly negative between hygiene scores and cow temperament, i.e., -0.19 between UHS and GT ($p< 0.05$), and -0.36 ($p< 0.001$) between LHS and GT. Moderate to strong significant correlations were observed between temperament traits, i.e., between AGG and IHRO (0.36; $p< 0.001$).

Table 4: Pearson correlation of subjectively scored welfare assessment traits (WAT) and temperament traits.

	BCS	LS	UHS	LHS	IHRO	AGG	GT
BCS	1	0.08 ns	-0.11 *	-0.10 ns	-0.19 *	-0.08 ns	0.15 ns
LS		1	0.02 ns	0.07 ns	0.03 ns	-0.02 ns	-0.1 ns
UHS			1	0.65 ***	-0.20 *	0.12 ns	-0.19 *
LHS				1	-0.25 *	0.09 ns	-0.36 ***
IHRO					1	0.36 ***	-0.04 ns
AGG						1	-0.13 ns

*= $p < 0.05$, **= $p < 0.01$, ***= $p < 0.001$, ns= not significant

AGG= Aggressiveness; GT= General Temperament; BCS= Body Condition Score; LS= Locomotion Score; IHRO= Intra Herd Rank Order; UHS= Udder Hygiene Score; LHS= Leg Hygiene Score

Influence of sensor traits on production traits

Results from model (1) are depicted in Figure 10. Comparing different lying percentages of DSN cows within 24 hours, it was observed that higher milk yields (18.15 kg/d) were recorded for cows that lied down or rested less than 22 % a day. Subsequently, cows from the 'High' NA class showed significantly ($p< 0.001$) reduced milk yields of 15.36 kg/d. DSN cows from the 'Low' NA class (limited lying and resting frequency within 24 hours) showed higher, but not significant, milk yields, than their herd contemporaries from the 'Medium' NA class. Hence, increasing daily percentages in sleeping/lying reduces the time that is available for FEED and RUM. This finding corresponds with higher daily FEED ($p< 0.001$) and RUM percentages for higher yielding cows. DSN from the 'High' FEED (> 23 %/d) and 'High' RUM (> 41 %/d) class had highest milk yields with 18.98 kg/d and 17.47 kg/d, respectively. Similar observations were made regarding the influence of NA, FEED and RUM on Fkg, due to the close correlation between Fkg and Mkg. Thus, cows with low values for NA had higher Fkg (0.76 kg/d) compared to cows with high NA (0.66 kg/d). Consequently, cows with higher daily feeding and rumination percentages recorded greater Fkg (0.83 kg/d, 0.73 kg/d).

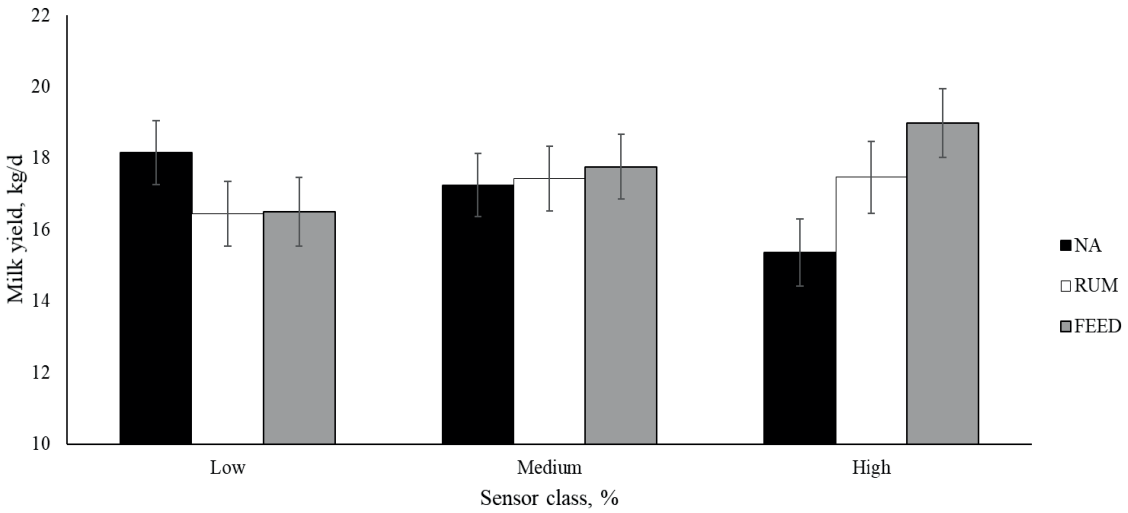

Figure 10: *Least square means and standard errors of milk yield of dairy cattle grouped into different sensor classes ('Low', 'Medium', 'High') for the traits not active (NA), rumination (RUM) and feeding (FEED).*

Influence of test-day traits on sensor traits

Results from model (2) are depicted in Figure 11, illustrating the FEED and RUM behaviour in dependency of Mkg and SCC classes. Although comparisons were not

significantly different, a trend of increased RUM percent per day for DSN cows with higher Mkg was observed. Similarly, FEED percent of cows increased with higher Mkg classes. Thus, cows with lower milk yield (<15-17 kg/d) spent significantly less time feeding (16.15-18.63 %/day) compared to cows from the high Mkg class (>25 kg/d= 20-25 %/d).

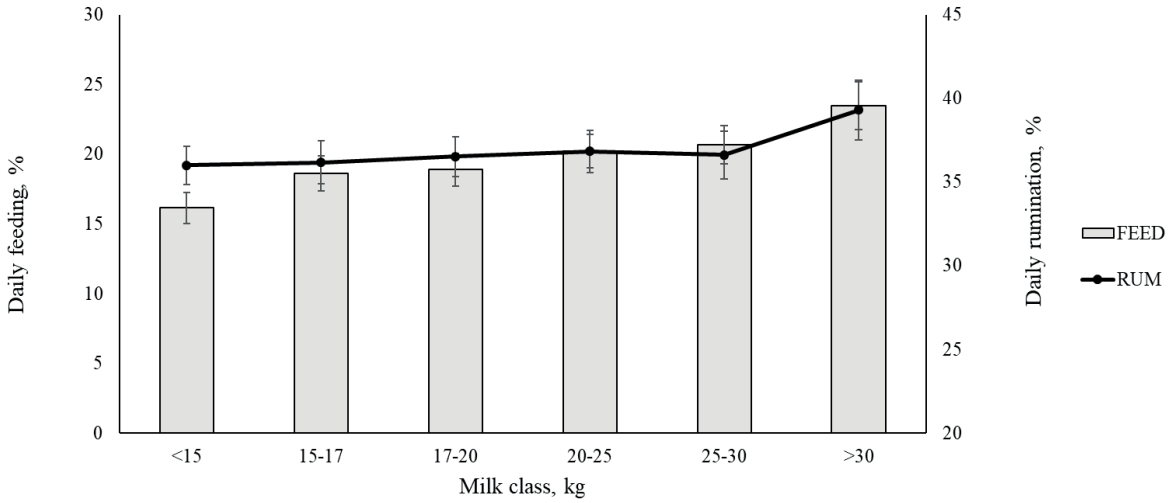

Figure 11: *Least square means of the sensor traits daily rumination (RUM) and feeding (FEED) percent and standard errors of dairy cattle grouped into milk yielding classes (kg).*

Regarding the fixed effect of SCC classes on sensor traits, opposite effects compared to the Mkg influence on FEED and RUM were found (Figure 12). While RUM remained on a quite constant level between 35.95 % and 38.36 % per day across the SCC classes < 50,000 – 700,000 cells/mL, a significant decrease of RUM (33.05 %/d) behaviour was obvious for DSN cows with an elevated SCC larger than 700,000 cells/mL. A similar trend was identified for FEED. Across the SCC classes < 50,000 – 350,000 cells/mL, the daily FEED percentage ranged between 18.70 % and 20.51 %. For cows with SCC > 350,000 cells/mL, the daily FEED percentage ranged within 15.91 % and 17.21 %.

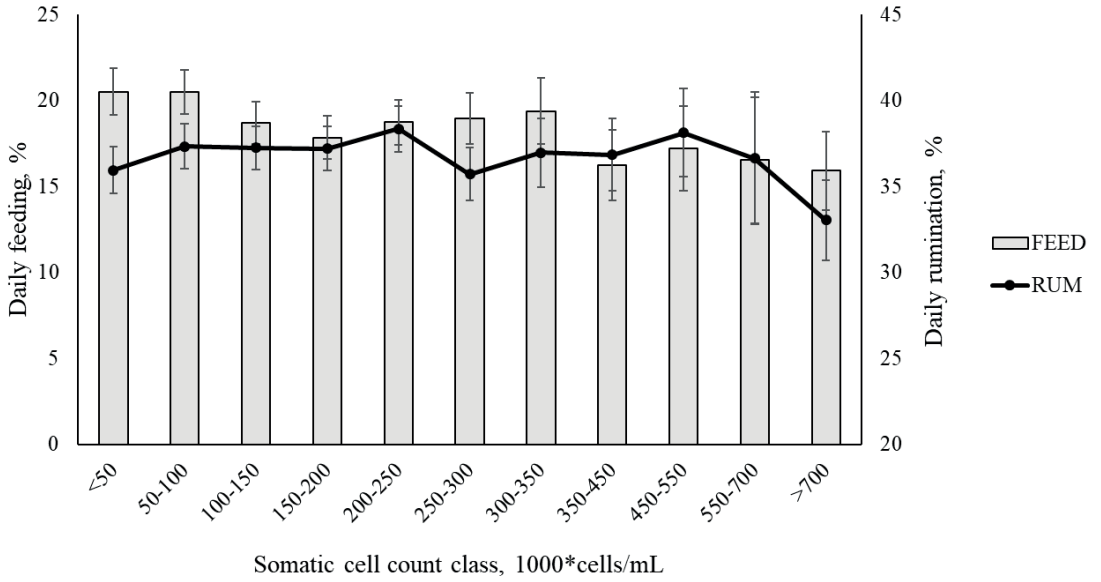

Figure 12: *Least square means of sensor traits of daily rumination (RUM) and feeding (FEED) percent and standard errors of dairy cattle grouped into distinct somatic cell count classes*1000 (cells/mL).*

Influence of parity and DIM on sensor traits

The results from model (2) showed a significant effect of parity (p> 0.01) on the daily FEED percentage. With increasing parity number, the daily FEED percentage significantly decreased from 21.20 % in the first lactation to 16.69 %/d in the fourth lactation. Although the effect of DIM was not significant, a decrease in daily FEED was observed from 18.72 % within the first 150 days down to 17.06 %/d in late lactation for DIM> 250. Output from the same model (2) showed decreasing RUM percentages from 38.71 %/d in the first parity to 33.56 %/d in the fifth parity. In contrast to FEED, we identified no significant difference between the daily RUM percentages in DIM dependency. Rumination was quite constant throughout lactation (<150 DIM= 36.84 %/d, 150-250 DIM= 36.09 %/d, >250 DIM= 37.11 %/d).

Influence of sensor traits on WAT

Mixed model analyses (model 3) showed significant impact (p< 0.001) of ACT, HA and NA on BCS (Figure 13). Locomotion score, LHS and UHS did not depict distinct differences in dependency of sensor trait classes. An increased BCS (3.7) was recorded within the lowest ACT class (<8 %/d). With increasing daily ACT, the BCS significantly (p< 0.001) decreased to a minimum value of 3.1 at the highest ACT class (> 12 %/d). Similar outcomes were detected for HA, where the BCS declined from 3.8 at the lowest

HA class (< 8 %/d) to 3.1 (p< 0.001) at the highest HA class (>11 %/d). Consequently, a reversed trend, but not significant, was found for NA. Hence, DSN cows in the lowest NA class (< 8 %/d) depicted a minimum BCS of 3.0, while the BCS significantly increased to 3.6 (p< 0.001) for cows in the highest NA class (> 25 %/d). The BCS increase from the first (BCS= 3.0) to the 'Medium' NA class (BCS= 3.2) was significant (p< 0.01). Overall, cows with reduced activity as reflected by low values for HA and ACT, had a larger BCS.

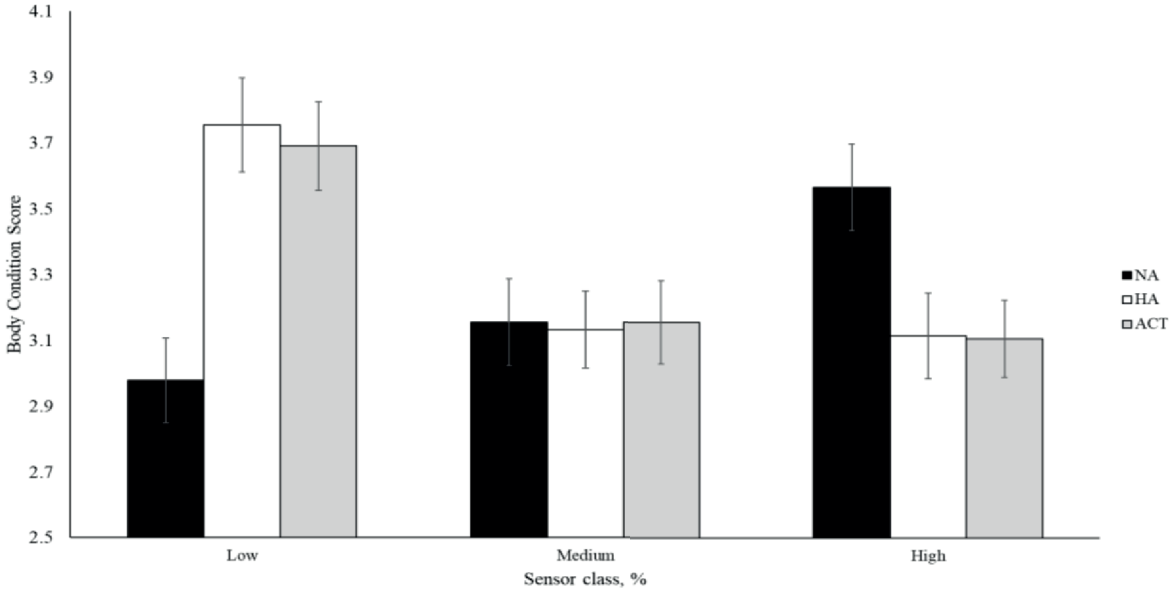

Figure 13: *Least square means of body condition score and standard errors of dairy cattle grouped according to sensor classes ('Low', 'Medium', 'High') of sleeping (NA), high activity (HA) and normal activity (ACT).*

Discussion

Correlations among sensor traits and between WTA and temperament traits

The negative correlation between ET and FEED implies that cows eat less with increasing core body temperature. Al-Kanaan (2016) showed, that an increasing temperature humidity index was associated with an increase of the skin as well as rectal temperature of dual-purpose DSN cattle. Thus, heat stress suppresses appetite in dairy cattle, which is their coping mechanism in avoiding additional release of heat from metabolic processes (Coffee, 1981). This is reflected by a reduced feed intake, slower fractional rate of digesta passage in the gastrointestinal tract, ruminal activity and motility (Silanikove, 1992). Further significant negative correlations among RUM, FEED, HA, NA and ACT depicted

the associations of behaviour traits among each other, due to the recording mechanism of the sensor. As the system only assigns one activity at a time, the percentage of the remaining four activities decreases (auto-correlated effect).

Noteworthy, significant correlations between LHS and UHS corroborated well with significant associations between udder and lower leg hygiene as described by Reneau et al. (2005). Moreover, moderate negative correlations between UHS with IHRO (-0.20; $p< 0.05$), and between LHS with IHRO (-0.25; $p< 0.05$) indicate that dominant cows are less dirty around legs and udder than submissive cows from the same herd. One possible explanation could be that lower ranking cows are left to use the least desirable places in the barn, which often show faecal contamination. In comparison, dominant cows occupy cleaner lying areas within the barn (Friend and Polan, 1974). Additionally, it was shown that submissive cows spent more time looking for bunk space during feeding and rested less, as they were chased away by dominant cows more often (Lamb, 1975). Prolonged time spent in the alleyways might contribute to increased udder and leg contamination of submissive cows as well. A moderate positive significant correlation between AGG and IHRO appeared reasonable, as particularly aggressive behaviour of dairy cattle determines the social rank in a herd (Lamb, 1975). Moderate negative correlations between UHS with GT, and between LHS with GT, indicate that calmer cows were less contaminated than nervous cattle. It can be argued that nervous animals were possibly hypersensitive to stress, leading to rapid movement through the barn, causing splashes which contaminate their udder and legs. Bartussek et al. (2002) linked cleanliness of the barn and of the cows directly to cow welfare, as it reflects the extent with which the stockman meets his responsibility for providing a well-managed housing environment and preventing damage and stress to the animals.

Influence of sensor traits on production traits

The different percentages DSN cows spend resting within 24 hours indicated that long resting periods reduce the time that is available for FEED and RUM, which in turn affects milk yield. Dual-purpose cows with large daily FEED and RUM percentages showed highest levels of milk and fat yields ($p< 0.001$). In support of our findings, Soriani et al. (2013) described positive relations between rumination time and milk yield as well. Moallem et al. (2010) observed, that a depression of rumination time led to a reduction

of DMI, followed by a decline in milk yield. Azizi et al. (2009) pointed out the importance of feed intake of dairy cows in order to maintain a high milk production level and recorded significant differences in DMI (meal sizes) between milking groups. Cows with high milk yield ate approximately 1.2 kg dry matter per meal more than cows in low production classes, while the feeding rate of multiparous cows exceeded the feeding rate of primiparous cows by 30 g DM/min. The results clearly state differences between sensor behaviour regarding FEED and RUM, that allow predicting a cow's production level. Moreover, changes in FEED and RUM behaviour can be used to identify distressed and sick cows more easily. The clear relation between a dual-purpose cow's production level and her daily behaviour pattern, suggests utilisation of the sensor system as a suitable early alert system for detecting physiological changes.

Influence of Mkg on sensor traits

The effect of production level on sensor traits (Figure 11) revealed, that DSN cattle in the highest milk yielding class (> 30 kg/d) recorded maximum RUM percentages (39.31 %/d). Stone et al. (2017) also corroborated positive associations between milk yield and rumination time in Holstein, Jersey and crossbreds (Holstein x Jersey). They argued that with increasing milk production, higher feed intake is required, implying increasing rumination times. In the present study, DSN cows with milk yields < 15 kg/d showed a minimum daily RUM of 35.99 %. While RUM remained steady between 36.17-36.83 %/d, it rapidly increased, once DSN cows passed the threshold of milking 30 kg/d (RUM= 39.31 %). In dairy breeds, a broad range for rumination duration per day has been reported. Braun et al. (2015) described daily rumination times of 368 ± 54 minutes (~25.52 %/d) for Brown Swiss cattle with a daily milk yield of 28.4 ± 2.9 kg at 4.4 years of age and 12.5 weeks postpartum. In contrast to this grazing-system research, it was a study conducted in an indoor system. In the indoor system, hay was available ad libitum during the day; corn silage was fed several times during the day and concentrate rations depended on the level of production. DeVries et al. (2009) reported an average rumination time of 555 min/d (~38.5 % RUM) in mid-lactating HF cows fed a 60:40 forage:concentrate ratio at a milk production level between 34-40 kg/d. Hence, HF demonstrated a much higher production level, but rumination times were similar in comparison to DSN. Apart from physiological breed differences, these discrepancies may be due to varying feeding regimes (DSN: grazing, HF: indoor system). Dado and Allen

(1996) found that a 10 % increase of natural detergent fibre prolonged rumination by 130 min/d. The DSN cows from our study were fed roughage and grass silage without receiving concentrates. The higher percentage of roughage and natural detergent fibre in the ratio might explain the increased daily RUM percentages in DSN. The slight increase in RUM beyond 250 DIM from 36.48 % to 37.6 % is in agreement with results by Miguel-Pacheco et al. (2014).

A similar trend to RUM was delineated for FEED in relation to different milk yield classes (Figure 11). Daily FEED significantly differed ($p< 0.001$) between the lowest and the highest milk yield class, confirming increasing feed intake with increasing milk production (Stone et al., 2017). In general, the feeding percentage for DSN was slightly lower than for HF. For example, DeVries et al. (2009) assigned 395 min of daily feeding time (~27.4 %) for Holstein cattle. In contrast, Braun et al. (2015) recorded similar ranges of feeding times for HF (206-207 min/d; ~14.3 %-21.3 %), Brown Swiss (262-338 min/d; ~ 18.7-23.43 %) and Swiss Fleckvieh (207-309 min/d; 14.4-21.4 %). As a corollary, it can be stated that the sensor system detected small differences in the daily FEED and RUM behaviour of dual-purpose cows, kept in pasture-based production systems, compared to indoor managed Holstein cattle. These distinctions are most likely due to differences in the physiology of dual-purpose and high yielding breeds regarding their metabolism and energy requirements, as well as due to variation in feeding and management.

Influence of SCC on sensor traits

Figure 12 illustrates the physiological impact of the udder health indicator SCC on DSN FEED and RUM behaviour. A significant difference ($p< 0.05$) in FEED and RUM behaviour was found between DSN cows with 200,000-250,000 cells/mL and cows with >700,000 cells/mL. In the study by Soriani et al. (2012), a five percent drop in daily rumination was due to fever caused by (sub-) clinical mastitis. Soriani et al. (2012) determined reduced rumen contractions as typical signs of clinical mastitis in dairy cattle. Chapinal et al. (2014) used rumination behaviour as an on-farm management tool for mastitis treatment efficacy monitoring. Also, for DSN kept in the grazing system, we found associations between SCC levels and daily FEED time (Figure 12). Feeding varied between 20.51 %/d and 17.85 %/d for SCC levels up to 350,000 cells/mL. A substantial

drop in daily FEED down to 16.24 %/d at 350,000-450,000 cells/mL might explain the physiological (sub-) clinical mastitis response. Significant differences ($p< 0.05$) for FEED between the SCC classes (<50,000 cells/mL and 150,000-200,000 cells/mL, <50,000 cells/mL and >700,000 cells/mL, 50,000-100,000 cells/mL and 150,000-200,000 cells/mL, <50,000-100,000 cells/mL and >700,000 cells/mL) underline the assumption of changing physiological behaviour. Noteworthy physiological changes in RUM and FEED were identified for elevated SCC levels at 350,000-550,000 cells/ mL. A relatively late physiological response of RUM to higher SCC might be due to the relation between immune competence and immunoregulators (Wellnitz et al., 2010). Wellnitz et al. (2010) concluded, that also very low SCC impaired the immune competence of udder quarters. Distinct relationships between the udder indicator trait SCC and daily behaviour were observed, i.e., a clear endogenous defence reaction towards a disease was reflected by sensor traits. A significant decrease in FEED was detected at a SCC threshold of 350,000 cells/ mL. For RUM, the reaction to increased SCC required a higher threshold, implying that FEED is more sensitive to diseases as RUM. Distressed DSN cows decrease their feed intake earlier than showing an obvious RUM response. Especially the sensor trait FEED might be a suitable indicator trait that can be considered as an early warning alert for mastitis under grazing conditions.

Influence of parity and DIM on sensor traits

Daily RUM percentages decreased with increasing parity number. RUM decreased from 38.71 %/d in the first parity to 33.56 %/d in the fifth parity. Similarly, FEED decreased from 21.2 %/d in the first parity to16.69 %/d in the fourth parity. One possible explanation could be that cows in higher lactations feed more efficiently, which means they realize a higher DMI in less time. In this regard, Azizi et al. (2009) described the “adaptation” potential of cows to increase DMI while reducing their overall feeding time. Dado and Allen (1996) related varying feeding rates to differences in body weight, energy demand for lactation and increased rumen fill of primiparous cows. In contrast to our results, Soriani et al. (2012) identified increasing rumination with increasing parities. Generally, pluriparous Holstein dairy cattle show higher levels of daily rumination than primiparous cows. Opposite results in our study might be due to breed differences or due to the specific grazing environment.

A significant FEED difference ($p < 0.05$) was observed between DIM. The highest FEED (18.91 %/d) during 150-250 DIM decreased to 17.06 %/d with DIM >250. The FEED decrease by DIM can be explained with the basic physiological processes underlying the lactation curve. For example, Norring et al. (2012) related changes in feeding behaviour to decreasing energy requirements due the milk yield decline. Applying the sensor technology allows further cow management optimisations, concerning the impact of parity and lactation stage on dual-purpose FEED and RUM behaviour. Such sensor information supports the farmer to individually adjust the management and feeding regime to lactating cows. Thus, also the sensor trait variations suggest grouping DSN cows according to their lactation number, production level or lactation stage, to better meet their energy requirements.

Influence of sensor traits on WAT

Sensor traits were weakly related with UHS, LHS and LS. An increase of LS, reflecting lame cows, did not result in significantly decreased ACT, HA or increased NA. This implies, that claw diseases in dual-purpose DSN are unlikely to be detected by sensor at an early stage. This might be a particularity of the cow friendly grazing environment. Also, from the background of a grazing environment, increased daily activity or lying periods did not significantly affect the leg or udder cleanliness of DSN cows. High BCS were recorded for cows with reduced daily ACT and 'High' NA (Figure 13). Subsequently, these cows spent more time resting, than with FEED or RUM. We showed that high-yielding cows spent more time feeding and ruminating than being inactive. In consequence, these cows had a lower BCS, due to their extensive fat mobilization to maintain enough energy for a higher milk production. In contrast, low yielding cows do not spend as much time feeding and ruminating, but resting, revealing a higher BCS. This leads to the classification of two general cow types within the research herd: One group spends more time feeding, ruminating and being active with a generally lower BCS, and a second group with increased BCS but reduced activity, feeding, rumination and lower milk yield. Roche et al. (2009) corroborated these findings, implying that body condition loss correlated positively with cow activity and peak milk yield. Garnsworthy and Topps (1982) analysed the effect of disparate calving BCS on milk production, feed intake and early lactation BCS change. They identified a negative effect of calving BCS on milk yield, with leaner cows producing more milk, because of a larger DMI. Reduced feeding

activity could also be a consequence of over conditioning (Garnsworthy and Topps, 1982). Hence, the sensor trait ACT can be associated with BCS.

Conclusion

The behavioural observations via visual observation as well as via innovative sensor technology contributed to a better understanding of dual-purpose cow behaviour under grazing conditions. Hence, automatically recorded longitudinal sensor traits can be used to improve grazing cow management and preventive health management. In this regard, cows with increased daily feed intake and rumination showed lower SCC. Moreover, it was demonstrated that feeding could be used as an indicator trait for early mastitis detection as a decrease of daily feeding percent was already noticeable at 350,000 cells/mL. Changes in rumination were also detected in case of mastitis, however here a much higher SCC threshold of 700,000 cells/mL was required, making this trait less sensitive to mastitis infection than feeding. Furthermore, an increase in daily resting indicated a lower milk yield production while high daily rumination and feeding percentages were associated with a high milk production potential in DSN. The sensor behaviour data also offered valuable insights to energy requirements in dual-purpose cows within different parity and lactation stages. Although net energy lactation in dual-purpose cows may not be as high as for Holstein Friesian cows, significant differences in feeding time per day were found for high milk yielding DSN cows. For the welfare assessment traits, a relation between BCS and the DSN daily activity was observed, while no distinct interrelations were found between sensor traits and hygiene scores or locomotion.

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CHAPTER 4

Multi-Breed GWAS for Electronically Recorded Cattle Behaviour Traits

Introduction

The fundamental interest of dairy farmers and breeders lies within understanding the role genetics play in the expression of animal behaviour and how it affects their productivity. Behaviour can be described as an essential part of biological regulations and as an integral effect influencing animal production and welfare. A better understanding of genetic animal behaviour mechanisms allows selection of dual-purpose cattle, which are well adapted to current and future production systems or which show an improved feeding behaviour (e.g. higher dry matter intake). Furthermore, welfare aspects can be derived from cattle behaviour. Hohenboken (1986) listed traits of cattle, which are known to be partly under genetic control, such as feeding, reproduction, social interaction and temperament. Additional findings about ruminants revealed, that a proportion of variation in foraging behaviour is genetically inherited (Launchbaugh et al., 1999; Snowder et al., 2001). This enables a selection for behavioural attributes, associated with feed intake. Next to feeding, rumination time and number of rumination intervals are considered novel traits, which are associated with milk yield and butterfat production.

Schutz and Pajor (2001) discovered substantial variation in behavioural traits, which in many cases, were favourably correlated with production traits. Although a better knowledge about how traits influence dairy cattle habits may be of high economic value, little is known about the genetic control of their expression (Schutz and Pajor, 2001). In general, cattle behaviour traits are considered low to moderate inheritable, with heritabilities ranging from 0.01 to 0.44 (Løvendahl and Munksgaard, 2016; Byskov et al., 2017). Furthermore, quantifiable phenotype data and single nucleotide polymorphism (SNP) marker information is required, to implement genome-wide association studies (GWAS) and to unravel genetic architecture of complex traits, that are involved in cattle behaviour.

Currently, the status of cattle breeding on a molecular level, regarding behaviour traits, is at its very beginning. So far, no candidate genes were significantly associated with cattle behaviour traits. This emphasizes the complex underlying genetic as well as molecular basis of quantitative traits, the importance of environmental effects and the need for broad phenotypic data recording, characterizing the behaviour (Schutz and Pajor, 2001). Although, understanding the genetic background of cattle is desirable, in order to improve

animal wellbeing and production, published studies suggesting genes, that affect cattle temperament, feeding and reproductive behaviour are scarce (Adamczyk et al., 2013). Alam et al. (2012) proposed, the polymorphism of bovine neuropeptide Y5 receptor gene (*NPY5R*) might potentially modify the action of neuropeptide Y and affect the regulation of appetite and feeding behaviour in beef cattle. Results generated from Korean Hanwoo cattle corroborated similar findings on the polymorphism of the melanocortin 4 receptor gene (*MC4R*), affecting feed intake capacity and feeding behaviour (Seong et al., 2012).

Since the beginning of animal husbandry, the influence of domestication and implementation of breeding programs led to extensive linkage disequilibrium (LD) within the bovine genome (e.g. LD> 0.3 at 250 kb). Consequently, confidence intervals of the significant SNP are long, indicating the difficulty of precisely mapping potential candidate genes (Raven et al., 2014). Raven et al. (2014) hypothesized, that less LD across bovine breeds and thus, a multi-breed GWAS could more accurately pinpoint the location of well-described mutations. By including different breeds, LD over short distances (5-10 kb for *Bos taurus*) rather than long-range LD could be conserved (De Roos et al., 2008; Raven et al., 2014). In other words, the SNP would have to be located in close proximity to the quantitative trait loci (QTL), in order to have an effect across multiple breeds. This way, effects, associated with SNPs, are less likely to derive from relationship within a breed and are rather due to LD with the QTL (Habier et al., 2010; Roos et al., 2008). This phenomenon is well exploited in refining QTL regions in dogs to a range of ~100 kb, which only contain limited candidate genes (Sutter, 2004). First, a dog breed with extensive LD is used to identify QTL, in a second step, multiple dog breeds and dense SNP typing are used to precisely map causal variants (Raven et al., 2014; Sutter, 2004). De Roos et al. (2008) requested, that around 300,000 SNPs should be included to keep the persistence of LD between divergent breeds, such as Angus and Jersey. Hence, for QTL, that segregate in multiple breeds, multi-breed GWAS could result in more precise mapping, while within breed analysis contributes to a better detection power of identified QTL (Raven et al., 2014). In addition to a higher GWAS resolution, a multi-breed GWAS is likely to identify older, conserved mutations, but may fail to effectively detect recently diverged mutations (Raven et al., 2014). As the aim of this study is, to investigate the

cause of genetics in basic bovine habits, a multi-breed GWAS seems to be the appropriate method to pursue.

The current study is based on multiple-breed genomic SNP data from four countries (Germany, Poland, Slovenia, Switzerland), including five different dual-purpose cattle breeds. This is the first study conducting a multi-breed GWAS on continuously recorded 24 h behaviour data of loose-housed dual-purpose cattle. The overall assumption is, that intensively recorded longitudinal electronically data of basic cow behaviour (feeding, ruminating, resting, activity, high activity) and ear temperature across different herds might reveal associated makers within the bovine genome. Thus, it is hypothesized, that it is very much likely, that dual-purpose breeds share ancestral mutations and recombination events can be used to map QTL to a smaller genomic interval and to identify significant SNPs. In this regard, Gutiérrez-Gil et al. (2015) have shown, that in many cases selection signatures are also shared by breeds with different production characteristics. These may be regions of interest in relation to metabolic homeostasis, or other general traits, such as disease resistance and behaviour (Gutiérrez-Gil et al., 2015). The objectives of this study were i) to identify the ancestry via allele sharing distances and to cluster European dual-purpose and dairy cattle breeds into groups; ii) to estimate genetic parameters for behaviour traits, based on pedigree and genomic information; and iii) to detect associated SNPs and potential candidate genes, underlying general cattle behaviour across different European dual-purpose breeds.

Materials and Methods

Phenotypes

Phenotypic records of five dual-purpose cattle breeds from Germany (DE_DSN= black and white dual-purpose cattle), Poland (PL_BS= Brown Swiss, PL_HF= Holstein Friesian), Slovenia (Sl_BS= Brown Swiss, Sl_Si= Simmental) and Switzerland (CH_OBS= dual-purpose original Brown Swiss, CH_Si= Simmental) were combined, in order to perform a multi-breed GWAS (Table 5). In Germany and Poland animals originated only from one research farm. The Slovenian data was recorded at three farms and in Switzerland, animals came from one original Brown Swiss herd and from one Simmental herd, located at another research farm. All farms can be characterized as

pasture-based production systems, which granted the animals a daily pasture access for at least 6 hours from May until November. Herd sizes ranged from 24 to 250 cows. The German research farm is located in the federal state of Hessen with 190 m above sea level. The two Swiss research farms are located at an elevation of 500 m and 1.100 m above sea level 40 km and 90 km south of Basel. The Polish farm can be found 80 km away from the Baltic Sea at 20 m elevation. All three Slovenian farms are located in mountainous regions west of Slovenia at 920 m to 970 m above sea level.

Table 5: Phenotype data of cattle breeds included in multi-breed GWAS and genetic parameter estimation.

Country	Breed	Sensor animals	Sensor animals with genotypes	Sensor records	Sensor records per cow
DE	DE_DSN	69	46	22,718	329.25
PL	PL_BS	49	28	17,332	353.71
	PL_HF	66	51	24,386	369.49
Sl	SI_Si	17	14	2,973	174.88
	SI_BS1	20	20	3,617	180.85
	SI_BS2	8	8	1,633	204.13
CH	CH_OBS	45	36	11,944	265.42
	CH_Si	45	43	12,446	276.58

DE_DSN= black and white dual-purpose (Germany), PL_BS= Brown Swiss (Poland), PL_HF= Holstein Friesian (Poland), Sl_Si= Simmental (Slovenia), Sl_BS= Brown Swiss (Slovenia), CH_OBS= original dual-purpose Brown Swiss (Switzerland), CH_Si= Simmental (Switzerland)

Sensor traits. In order to generate a longitudinal data structure of electronically recorded behaviour data, 249 sensors of the Dutch CowManager system (Agis Automatisering BV) were implemented in the European research herds, beginning in June 2016. After one month of adaptation, data from July 2016 until March 2018 of 319 cows was used for processing. Only records of cows with at least ≥30 consecutive days of sensor observations were included. Once implemented into the animal's left ear, the sensor system uses a 3-dimentional accelerometer (attached to an RFID tag), to match records of every minute to five behaviour categories, based on location parameters measuring different movement patterns (rumination, feeding, resting / non-active, moderate activity, high activity). Next to behaviour recordings, the sensor uses a digital surface temperature monitor to measure the mean hourly ear surface temperature (ET). The system detects rumination (RUM), based on the typical repetitive ear movement, caused by the chewing and regurgitation of the cow. Feeding (FEED) relates to the food intake, expressed through masticatory movement. The activity parameters are subcategorized into basic

activity (ACT), high activity (HA) and no activity (NA). The state of ACT describes any kind of moderate ear movement resulting from walking, head shaking or other, that cannot be associated with the repetitive ear movement during RUM or FED. High activity (HA) results from increased ACT, that in most instances is elevated prior to parturition or during oestrus, including mounting behaviour. No activity refers to a minimum or no ear movement, while the cow sleeps or rests. The hourly percentage of time spent for every behaviour is transmitted through a wireless connection to a router within the stable and at the end the hourly percentages were further transformed to daily time percentages. Whenever the sensor records a certain behaviour such as RUM, it does not assign this time to another behaviour trait (e.g. NA) (Agis Automatisering BV, Harmelen, the Netherlands). Additionally, to evaluate the five sensor behaviour categories, two more were formed: index (I) and index class (IC). The index was formed according to the welfare quality assessment protocol® (2009) (Table 6). Physiological thresholds of every sensor behaviour were assigned with a grade from 0 to 2 and summed up. The IC consisted of I, grouped into classes with additional conditions depicted in Table 7.

Production traits. Test-day records included the varied lactations (from 1 to 12) within the calving years of summer 2015 until winter 2018. Test-day milk yield (Mkg), fat percentage (Fat %), protein percentage (Pro %) and the log-transformed somatic cell count (somatic cell score= SCS) were available for 329 cows from Germany, Poland and Switzerland.

Table 6: Composed index of phenotyped animals with >30 days of sensor recordings, based on the welfare quality assessment protocol® (WELFARE QUALITY®, 2009).

	Rumination			Feeding			Active			High Active			Not Active		
	Min	Opt	Max	Min	Opt	Max	Min	Opt	Max	Min	Opt	Max	Min	Opt	Max
Range, %/d	< 29.2	29.2-41.7	> 41.7	< 12.5	12.5-20.8	> 20.8	< 8.3	8.3-12.5	> 12.5	< 8.3	8.3-12.5	> 12.5	< 16.7	16.7-41.7	> 41.7
Range, h/d	<7	7-10	>10	<3	3-5	>5	<2	2-3	>3	<2	2-3	>3	<4	4-10	>10
Points	0	2	1	0	2	1	0	2	1	1	2	0	1	2	0
Meaning	A	N	OK	A	N	OK	A	N	i.h.	-	N	i.h.	A	N	A

A= alarming (check animal or management), N= normal, ok=harmless, but not as good as N, i.h.= possibly in heat, Opt= optimum (normal) behaviour range, gathered from literature

Table 7: Composed index classes (from Index see table 6) of phenotyped animals with >30 days of sensor recordings, based on the welfare quality assessment protocol® (WELFARE QUALITY®, 2009).

Class	Meaning	Points	Criteria
1	Excellent	> 6 (7-10)	At least 1 point in every sensor trait category, FEED and RUM should have 2 points
2	Acceptable	5-9	
3	Poor (health/ welfare impairment)	< 5	

Genotypes

The five breeds, mentioned above, and two additional breeds from Germany (DE_DN= dual-purpose Red and White; DE_HF= German Holstein) were genotyped with the *Illumina BovineSNP 50 k Bead chip (v2 and v3)*, according to the Illumina Infinium assay protocol (Illumina Inc., San Diego, CA, USA). The quality control of the genotypic data was conducted applying the software PLINK (Purcell et al., 2007), defining a minor allele frequency (MAF) below 0.01 and a Hardy–Weinberg equilibrium of $p< 0.00001$. All SNPs had a call rate larger than 85 %, while excluding SNPs located on sex chromosomes. Cows with a call rate less than 80 % for all loci were also excluded. Whenever the relation between two animals was higher than 0.95, the animals were deleted. After SNP data editing, a total of 35,826 SNPs were available of 615 cows (Table 8). Sporadic missing SNP were imputed by the software BEAGLE version 3.3.2 (Browning and Browning, 2007).

Table 8: Genotype data of five cattle breeds included in PCA, WIDDE and multi-breed GWAS.

Country	Breed	No of animals	No of animals after quality control
DE	DE_DSN	266	266
	DE_DN	20	20
	DE_HF	50	50
PL	PL_BS	34	34
	PL_HF	59	59
Sl	Sl_Si	46	44
	Sl_BS	36	36
	Sl_HF	14	14
CH	CH_OBS	48	46
	CH_Si	48	46

DE_DSN= black and white dual-purpose (Germany), DE_DN= red and white dual-purpose (Germany), DE_HF= Holstein Friesian (Germany), PL_BS= Brown Swiss (Poland), PL_HF= Holstein Friesian (Poland), Sl_Si= Simmental (Slovenia), Sl_BS= Brown Swiss (Slovenia), Sl_HF= Holstein Friesian (Slovenia), CH_OBS= original dual-purpose Brown Swiss (Switzerland), CH_Si= Simmental (Switzerland).

Population structure and breed assignment

In order to account for potential population stratification and to explore the genetic diversity of the dataset, due to relatedness among the sampled individuals, a principal component analysis (PCA) was conducted prior to the GWAS. The PCA based on the genomic relationship matrix generated in GCTA (Yang et al., 2011). In a second step, a breed assignment analysis was conducted using the WIDDE program (Web-Interfaced Next Generation Database) (Sempéré et al., 2015). The WIDDE cattle data base contained

750,000 SNPs and 2,827 publicly available individuals, which belong to 129 different populations (Sempéré et al., 2015). The broad variety of local cattle populations are supposed to represent the bovine genetic diversity and cover the three main cattle groups, i.e., European and African taurine (*Bos taurus*) as well as zebus (*Bos indicus*) (Sempéré et al., 2015). The allele proximity between the own genotyped populations and the populations represented in the world reference dataset in WIDDE were estimated, based on supervised clustering (Sempéré et al., 2015). A stopping criterion of 0.01 was set in WIDDE, as a smaller value of criterion enhances the accuracy of estimated parameters (Sempéré et al., 2015). For each genotyped individual, the percentage of ancestry proportions between the individual and the 129 populations from the WIDDE world reference dataset was listed.

Statistical model for genetic parameter estimation

For the estimation of genetic parameters, a combination of genomic relationship matrix and pedigree (PGMIX procedure in DMU) (Madsen and Jensen, 2018), as well as only pedigree in a separate run, were included. The pedigree consisted of 8,798 animals and was traced back as far as possible. Variance components of sensor traits were estimated from univariate animal models using the AIREML procedure, as implemented in the DMU software package (Madsen and Jensen, 2018):

$$y=Xb+Z_1a+Z_2p+e \quad (1)$$

where y was the observation vector for sensor traits and indices (RUM, FEED, NA, ACT, HA, ET, I and IC); b was the vector of fixed effects, including breed-farm and measuring year-month, and age of the sensor cows is a fixed regression; a was the vector for additive genetic effects; p was the vector for permanent environmental effects; e was the vector of random residual effects, and X, Z_1, and Z_2 were incidence matrices for b, a, and p, respectively. It was assumed, that the variance-covariance structure of a ~ N (0, Kσ_a^2), where σ_u^2 was the genetic variance, K was the either genomic relationship matrix (G) or combined relationship matrix (H), when blending the pedigree-based relationship matrix (A) and weighted genomic relationship matrix (G_w) (Legarra et al., 2009; Christensen et al., 2012). G_w was calculated as follows:

$$G_w=(0.95\times G+0.05\times A_{22})$$

where A_{22} is the sub-matrix of the pedigree-based relationship matrix for genotyped animals. Estimated breeding values (EBV) from model 1, based on A matrix were de-regressed to produce de-regressed proofs (DRP) according to Garrick et al. (2009). Only animals with a DRP weight larger than 0.2 were kept in GWAS. Another repeatability model, using only A matrix, was applied on test-day production traits and SCS, respectively, to obtain the EBV and consequently to calculate the DRP. The classification fixed effects contained breed-farm and calving-year-season. The lactation curve, using Legendre polynomial three, on days-in-milk was also included in the model because test-day in varied lactations were included in the data. The three random effects in the model were the additive genetic effect, the permanent environmental effect and the residual effect.

Multi-breed GWAS

Single-trait multi-breed GWAS was performed, applying the software package GCTA (Yang et al., 2011) with the leave-one-chromosome-out (*loco*) option, using the mean of repeated sensor traits (MEAN) and DRP, calculated before as phenotypes. Testing single-locus SNP effects, the following statistical model was included:

$$y=Xb+Wg+Zu+e \quad (2)$$

where y was the vector of DRP or MEAN for RUM, FEED, NA, ACT, HA, ET, I, and IC); b was the overall mean, when using DRP as phenotypes, and breed-farm was included as a classification fixed effect, when MEAN were the phenotypes; g was the additive fixed effect of SNPs, tested for association; u was a vector of polygenic effects calculated based on all SNP, except those on the chromosome, where the candidate SNP was located; and e was the vector of random residual effects; X, W, and Z were incidence matrices for b, g, and u, respectively. In order to test the reliability of GWAS, based on small population size, DRP of test-day production traits and SCS were also analysed in model 2. The Bonferroni threshold was $p_{Bonf} = 0.05/35{,}826 = 4.47 \times 10^{-7}$. Next to the Bonferroni correction, the false discovery rate (FDR), introduced by Benjamini and Hochberg (1995), was applied as another significance threshold for genome-wide analysis. The FDR was set to 20 %, in order to detect candidate SNPs for behaviour traits.

Candidate gene annotation

The associated candidate genes were identified via the gene-based test in GCTA applying the *fastBAT* option. The database (version UMD3.1), including gene locations, start positions and end sites for every bovine gene was downloaded from Ensembl (Zerbino et al., 2018). Although, 24,616 gene ID entries were originally available in the data base, only 17,545 genes on chromosome 1 to 29 were included in further analyses, due to valid evidence in the gene ontology (Ashburner et al., 2000; Gene Ontology Consortium, 2017). At first, SNPs exploited in GWAS were mapped to the genes, applying a window of 50 kb upstream and 50 kb downstream from the genes. Subsequently, the output from annotations and SNP *p*-values from GWAS were used simultaneously, to detect genes, that were associated with sensor and production traits. Finally, the *p*-values of genes were adjusted according to FDR (Benjamini and Hochberg, 1995). For further understanding, physiological functions and positions of candidate genes were examined in the Ensembl (Zerbino et al., 2018), NCBI (Geer et al., 2010), UniProt (Chen et al., 2017) data bases.

Results

Population structure and breed assignment

PCA. When plotting the first and the second principal components (explaining 4.71 % and 3.05 % of variance), the PCA revealed two distinctly diverged clusters of genetic origin (Figure 14a). The Holstein lines showed obvious genetic differentiation from the other breeds (Sl_Si, Sl_BS, PL_BS, CH_OBS, and CH_Si). Depicting the first and third (explaining a variance of 2.38 %) principal components, three clusters were formed in a triangle-like 2-dimensional form (Figure 14b). Each cluster was positioned at the three apexes of the triangle, with the admixed populations of Sl_Si in a rather intermediate position. Three disparate groups were formed with DE_HF, DE_DSN, DE_DN, Sl_HF, and PL_HF on the left. A second cluster of PL_BS, Sl_BS and CH_OBS was located in the bottom right corner, and a third group in the top right corner, consisting of CH_Si and, slightly distanced Sl_Si. Illustrating the second and third principal components (Figure 14c), the three clusters mentioned above can also be seen according to the third principal component. However, the second principal component revealed the diversity within the Holstein breeds.

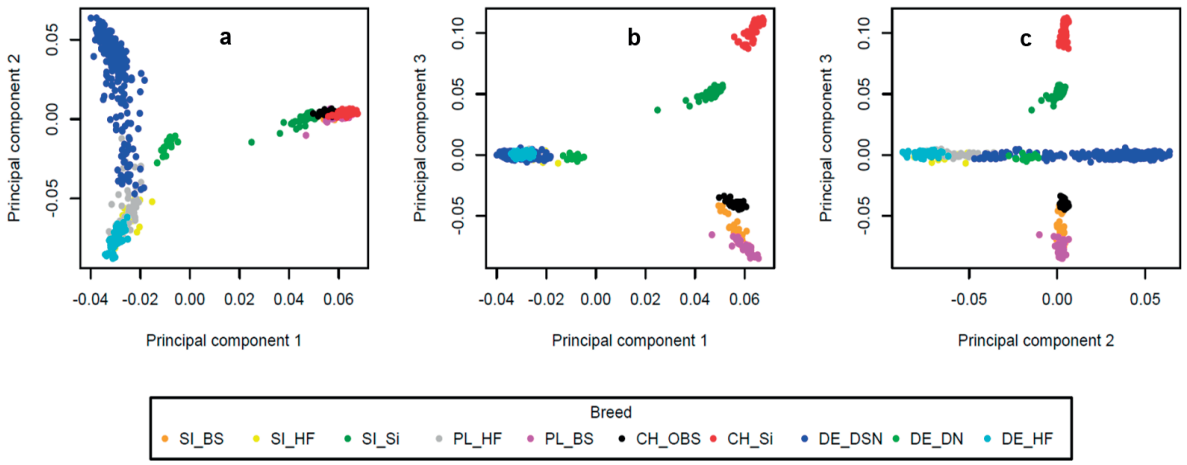

***Figure 14**: First three principal components, based on the genomic relationship matrix, generated in GCTA.*

WIDDE. The results of the breed assignment (Figure 15) illustrated ten populations, from the world reference dataset in WIDDE, with largest ancestry proportions to the uploaded populations. All breeds consisted at least to 57.83 % of European breeds, affirming their European origin. The predominant genetic affinities consisted of Holstein, Hereford, French Red Pied Lowland and French Brown Swiss breeds. However, aside from European ancestors, exotic ancestral proportions of Sheko, Zebu Bororo, Gir or Arabic Zebu appeared.

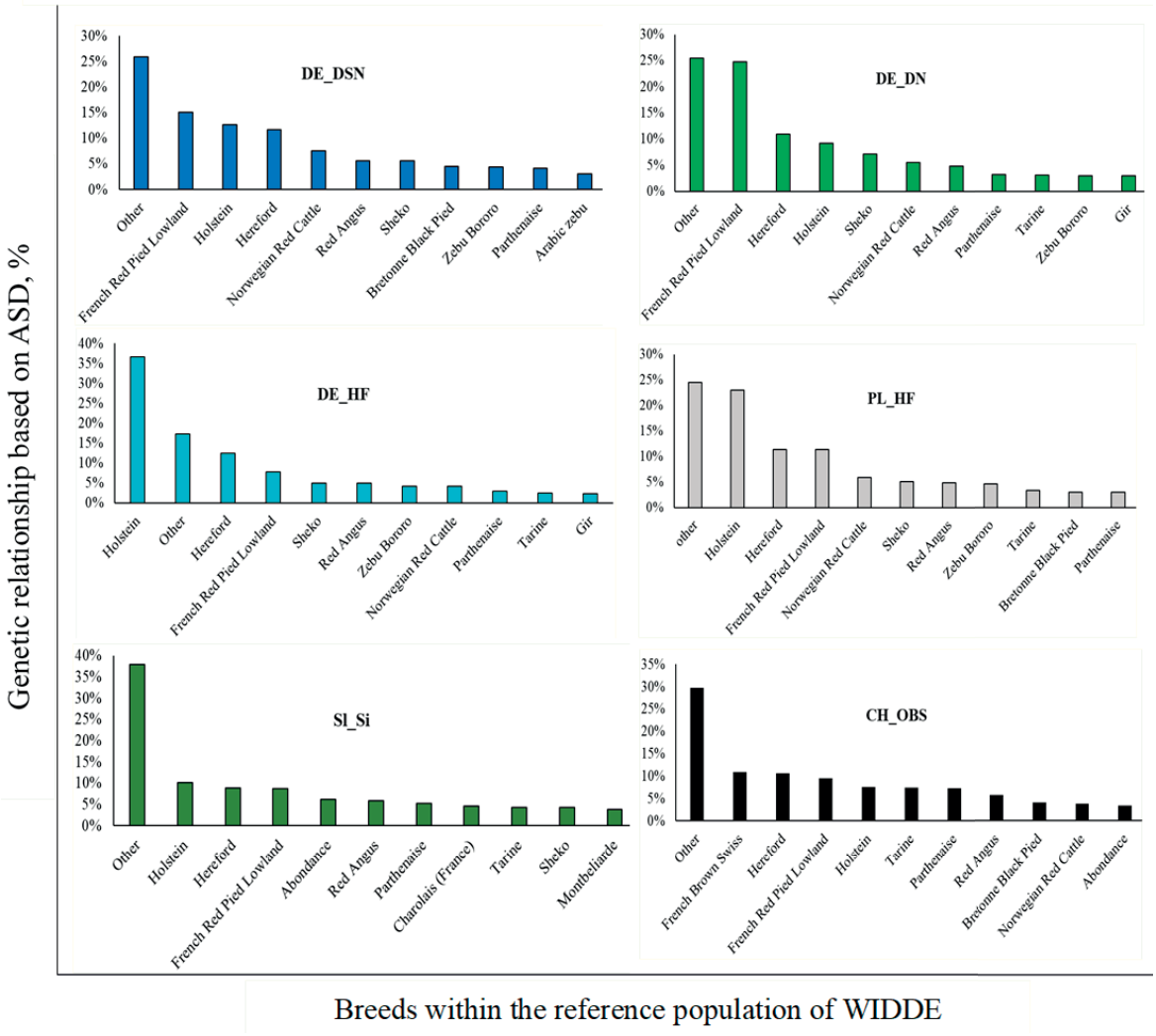

***Figure 15**: Ancestry composition of European genotyped animals, based on allele sharing distance (ASD).*

Genetic parameters of sensor traits

The descriptive statistics of sensor and production traits can be found in Table 9. The heritabilities of sensor traits, estimated via H or via A matrices, were very similar and were on a low to moderate level (Table 10). Standard errors (SE) of heritabilities were acceptable (< 0.06) and almost the same for the two relationship matrices. Rather moderate heritabilities (0.16-0.20) were found for FEED, HA and NA, which also showed quite large additive genetic and small residual variances. Low heritable traits (0.02-0.08), such as RUM, ACT, ET, I and IC had small additive genetic variances and large permanent environmental variances.

Table 9: Descriptive statistics of phenotypes of sensor and production traits.

Trait	No of observation	No of animals	MEAN	SD	Min	Max
RUM	97,049	319	34.13	7.07	5.94	81.36
FEED	97,049	319	23.87	8.47	0.19	66.32
ACT	97,049	319	8.45	5.28	0.16	50.75
HA	97,049	319	7.76	3.22	0.18	33.78
NA	97,049	319	25.79	7.51	4.58	72.83
ET	97,049	319	24.66	4.59	2.23	38.28
I	97,049	319	6.27	1.49	0	10
IC	97,049	319	2.04	0.42	1	3
Milk	6,571	329	19.33	6.3	1.6	47.2
Fat %	6,546	329	4.1	0.67	1.84	7.98
Pro %	6,546	329	3.43	0.41	2.12	5.5
SCS	6,546	329	2.43	1.54	-1.32	10.5

Table 10: Variance components (ϑ_a^2: additive-genetic variance, ϑ_{pe}^2: permanent environmental variance, ϑ_e^2: residual variance), reliabilities (r) and heritabilties (h^2) with standard errors (SE) for sensor traits of the cattle breeds: DE_DSN, CH_OBS, CH_Si, PL_BS, PL_HF, Sl_Si, Sl_HF.

	Pedigree						PGMIX (Ped+Gmatrix)					
	ϑ_a^2	ϑ_{pe}^2	ϑ_e^2	r	h^2	SE	ϑ_a^2	ϑ_{pe}^2	ϑ_e^2	r	h^2	SE
Rumination	1.00	10.31	29.59	0.28	0.02	0.04	0.84	10.52	29.59	0.28	0.02	0.05
Feeding	9.10	3.26	35.48	0.26	0.19	0.05	9.51	3.63	35.48	0.27	0.20	0.05
Active	1.98	4.53	18.47	0.26	0.08	0.05	1.57	5.04	18.47	0.26	0.06	0.05
High Active	1.55	0.72	6.01	0.27	0.19	0.05	1.68	0.73	6.01	0.29	0.20	0.05
Not Active	7.13	7.50	29.98	0.33	0.16	0.06	7.79	6.69	28.60	0.34	0.18	0.06
Temperature	0.86	2.10	9.21	0.24	0.07	0.04	0.84	2.19	9.21	0.25	0.07	0.04
Index	0.06	0.21	1.88	0.12	0.03	0.02	0.08	0.19	1.88	0.13	0.04	0.02
Indexcl	0.00	0.01	0.16	0.10	0.03	0.02	0.01	0.01	0.16	0.10	0.04	0.02

DE_DSN= black and white dual-purpose (Germany), CH_OBS= original dual-purpose Brown Swiss (Switzerland), CH_Si= Simmental (Switzerland), PL_BS= Brown Swiss (Poland), PL_HF= Holstein Friesian (Poland), Sl_Si= Simmental (Slovenia), Sl_BS= Brown Swiss (Slovenia), Sl_HF= Holstein Friesian (Slovenia)

Multi-breed GWAS

From the overall dataset, 35,826 autosomal SNPs were retained. The mean SNP coverage per 1,000 kb of 29 autosomes (BTA) was 14.2, ranging from 653 (BTA28) to 2,356 (BTA1) SNPs per chromosome, with a mean of 1,235 SNPs per chromosome. The SNP coverage was examined by dividing each autosome into windows of 1,000 kb. Ten chromosomes (BTA6, 7, 10, 12, 14, 15, 18, 21, 24, 26) depicted at least one window with a missing SNP. The pre-examination of the production trait fat percent depicted significant SNPs above the BF corrected threshold on BTA14 (Figure 16).

Overall, seven SNP markers, of MEAN and DRP of behaviour traits, were identified surpassing the FDR of 20 % (Table 11). Notably, one of the seven SNPs even remained above the more stringent BF corrected line. Significant SNPs were associated with the

behaviour traits NA, RUM and FEED (Table 11) and were distributed on six chromosomes. The most significant SNP was found on BTA13 (p= 2.36E-08) for the trait NA (Figure 17). For each trait, the number of significantly associated variants differed with five identified SNPs for RUM (BTA11, 17, 27, 29) (Figure 18) and one SNP each for NA (BTA13) and for FEED (BTA23) (Figure 19). For the remaining sensor traits, no significant SNPs were found (Figures 20-24). Most SNPs were detected when conducting GWAS using DRP of sensor traits rather than MEAN. Only in the case of NA, both procedures (MEAN, DRP) tagged the same SNP on BTA13 (Hapmap60738-rs29023086). The remaining significant SNPs were identified via DRP.

Table 11: SNPs associated with behaviour traits surpassing the false discovery rate (FDR) of 20 % or the Bonferroni corrected threshold (BF).

Trait	Chr	SNP	bp	SE	p	Threshold	Method
NA	13	Hapmap60738-rs29023086	79178395	0.83	1.07849E-06	BF	Mean
NA	13	Hapmap60738-rs29023086	79178395	0.79	2.35972E-08	FDR	DRP
RUM	11	BTB-01638234	55229674	0.35	2.04483E-05	FDR	DRP
RUM	17	ARS-BFGL-NGS-104430	68187177	0.61	1.79144E-05	FDR	DRP
RUM	27	ARS-BFGL-NGS-13449	37283994	0.42	1.35837E-05	FDR	DRP
RUM	29	ARS-BFGL-NGS-24800	46014507	0.35	9.06939E-06	FDR	DRP
RUM	29	ARS-BFGL-NGS-81862	49036580	1.29	2.00908E-05	FDR	DRP
FEED	23	ARS-BFGL-NGS-80066	19834215	1.13	5.12613E-06	FDR	DRP

RUM= rumination, NA= not active, FEED= feeding, Chr= chromosome number, bp= number of base pairs, DRP= de-regressed proof based on EBV output from DMU, FDR= False discovery rate 20 %, BF=Bonferroni corrected threshold

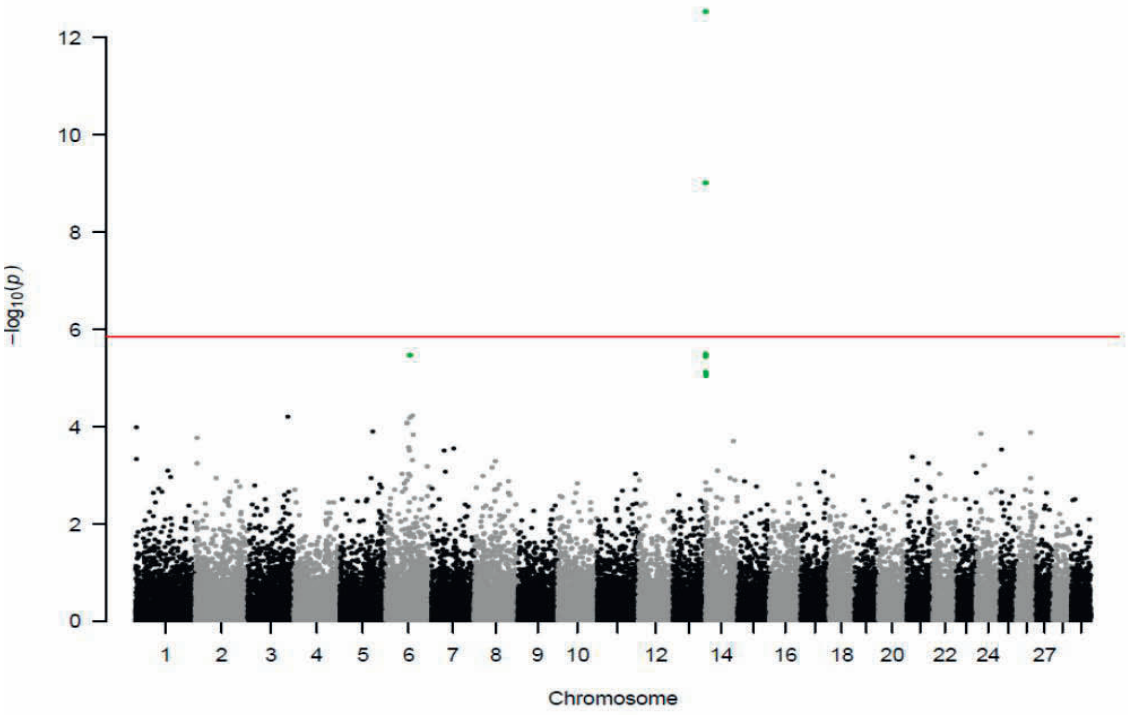

Figure 16: *Manhattan plot of the production trait fat percent with significant SNPs on BTA14.*

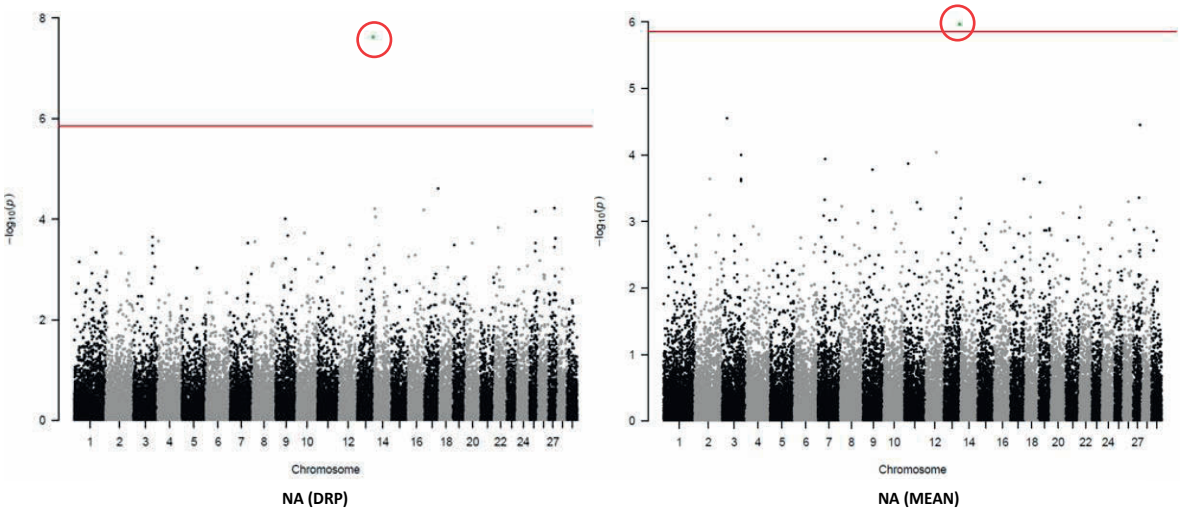

Figure 17: *Manhattan plots of the sensor trait Not Active (DRP, MEAN) with a significant SNP on BTA13.*

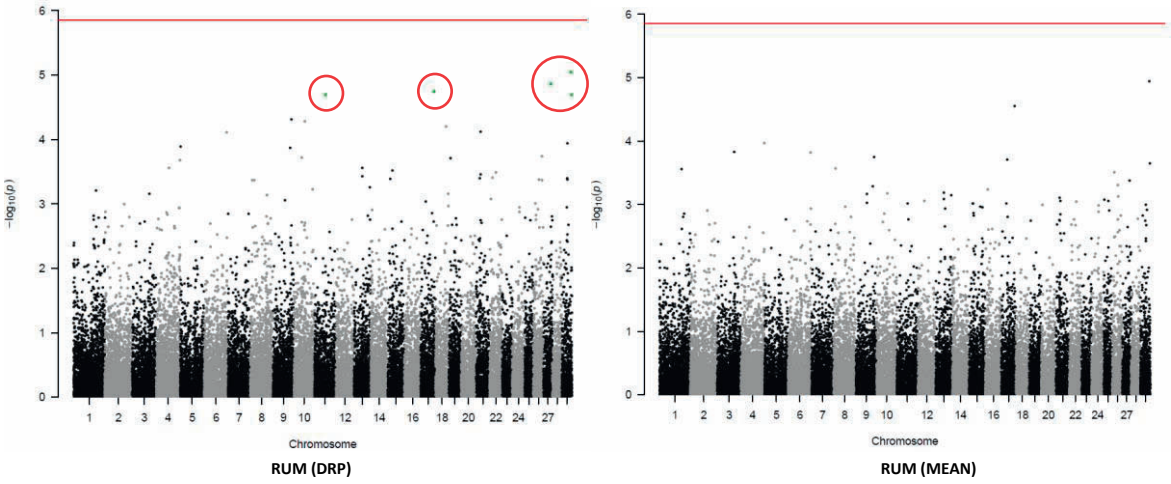

***Figure 18**: Manhattan plots of the sensor trait Rumination (DRP, MEAN) with significant SNPs on BTA11, 17, 27, 29.*

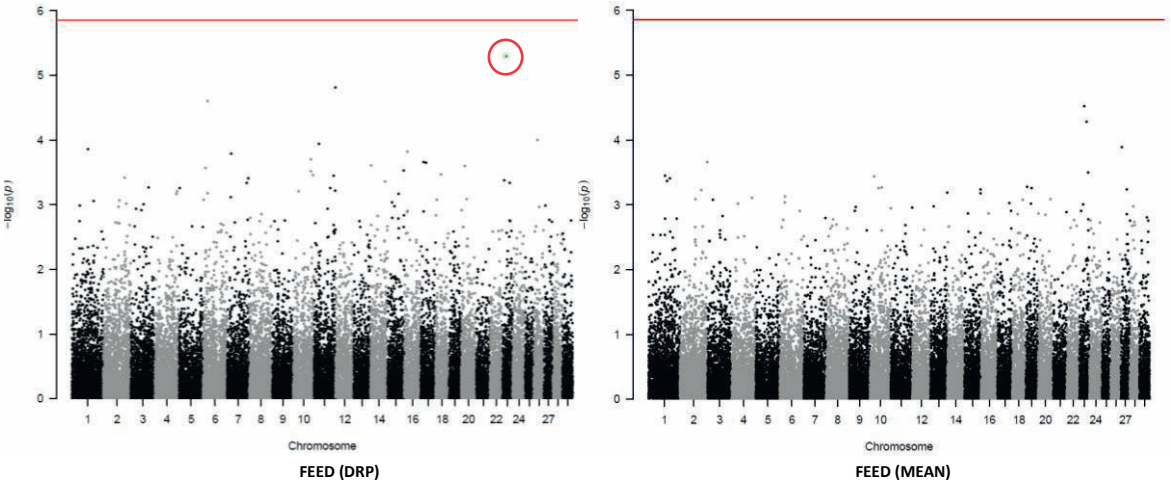

***Figure 19:** Manhattan plots of the sensor trait Feeding (DRP, MEAN) with a significant SNP on BTA23.*

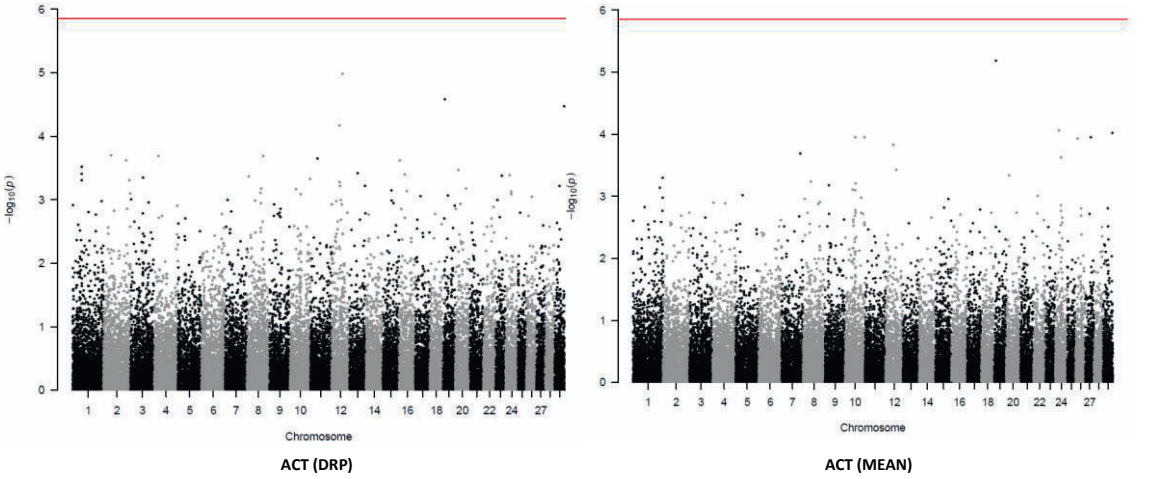

***Figure 20:** Manhattan plots of the sensor trait Activity (DRP, MEAN) without significant SNPs.*

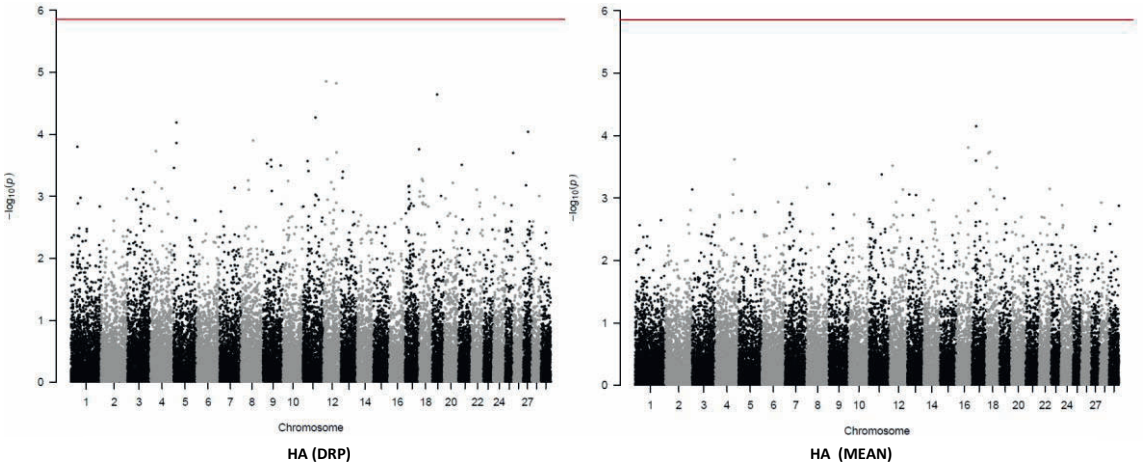

***Figure 21**: Manhattan plots of the sensor trait High Activity (DRP, MEAN) without significant SNPs.*

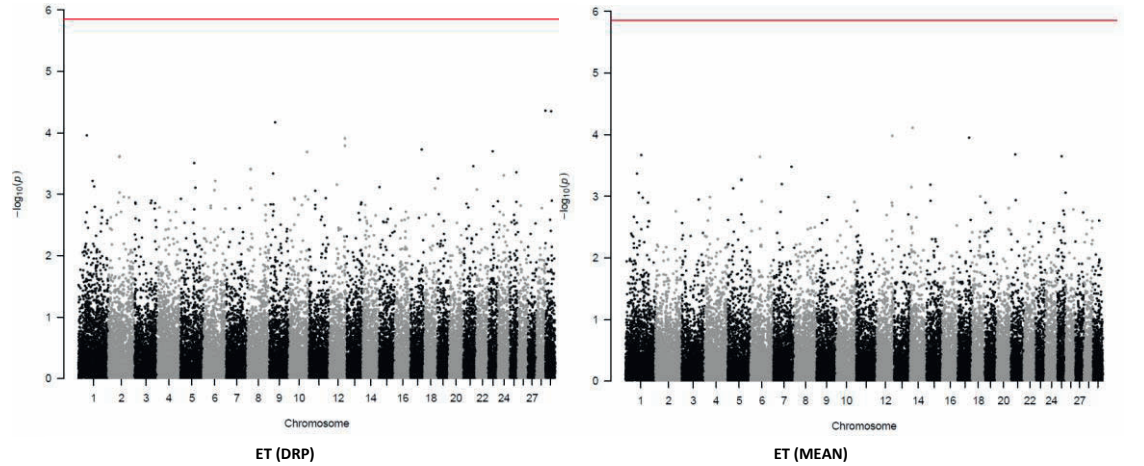

***Figure 22:** Manhattan plots of the sensor Ear Temperature (DRP, MEAN) without significant SNPs.*

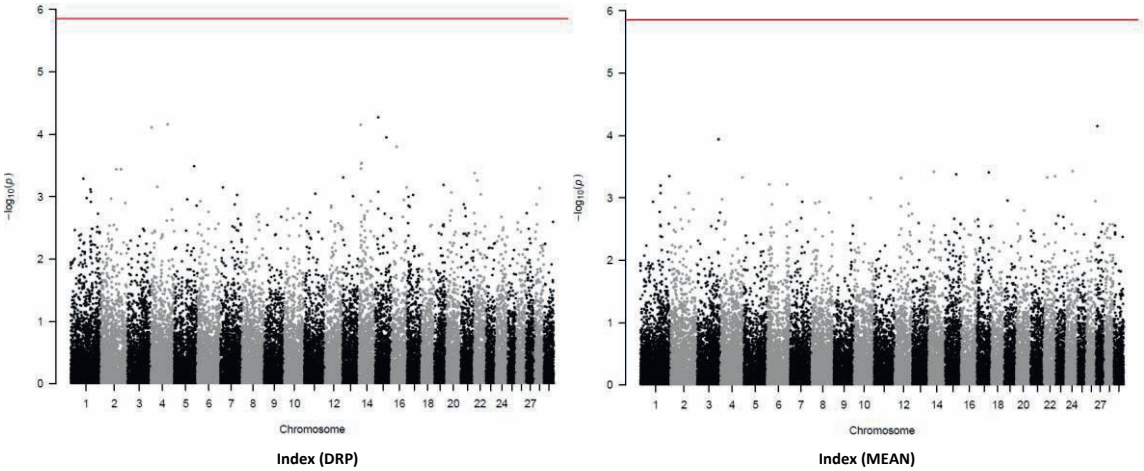

***Figure 23:** Manhattan plots of the combined sensor traits to Index (DRP, MEAN) without significant SNPs.*

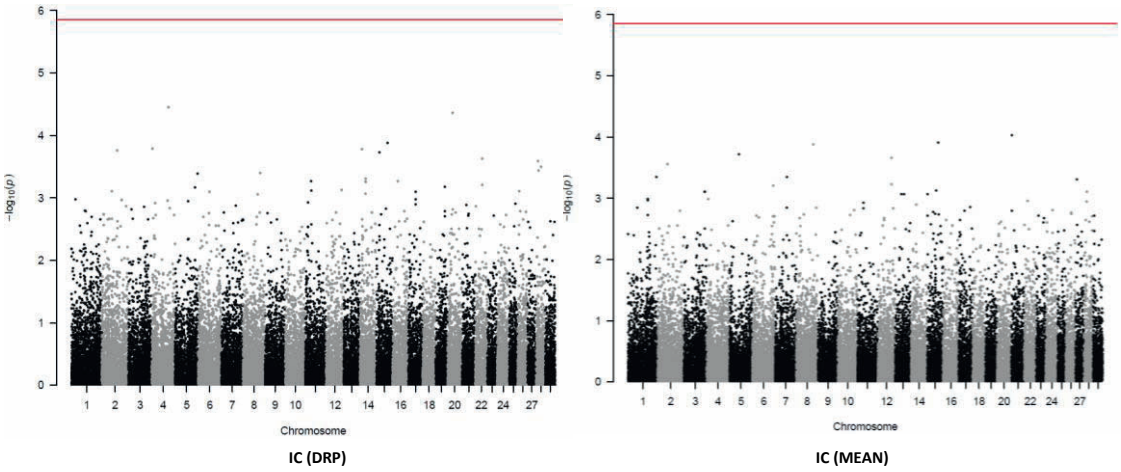

Figure 24: *Manhattan plots of the combined sensor traits to Index Class (DRP, MEAN) without significant SNPs.*

Identifying candidate genes within close proximity to significant SNPs the gene-based test, applying the *fastBAT* procedure, of GCTA was chosen and revealed 22 potential options (Table 12). These putative candidate genes might play a role in the expression of bovine behaviour in DE_DSN, PL_BS, PL_HF, Sl_Si, CH_Si and CH_OBS. The DRP and/or MEAN of RUM depicted five SNPs on BTA21, 27 and 29. In total 13 potential candidate genes were in near proximity to these SNPs of RUM. The SNP of the DRP of RUM on BTA21 was associated with the *BTBD1* gene. For the SNP of the DRP of RUM on BTA27 two possible candidate genes were found: *THAP1*, *RNF170*. The DRP as well as the MEAN for RUM revealed one SNP (ARS-BFGL-NGS-118751) on BTA29, which was associated with eight putative candidate genes (*RPS6KB2*, *PTPRCAP*, *CORO1B*, *GPR152*, *CABP4*, *TMEM134*, *AIP*, *PITPNM1*). The last two SNPs of the DRP of RUM on BTA29 were associated with a novel (*ENSBTAG00000000776*) and the *MRGPRG* gene. For the MEAN of the sensor trait ACT, one SNP was discovered (via *fastBAT*) on BTA19, which was associated with one putative candidate gene (*PPM1E*). The DRP of FEED revealed four SNPs on BTA11, that were in close proximity to seven potential candidate genes (*STXBP1*, *CFAP157*, *PTRH1*, *TOR2A*, *LCN8*, *LCN15*, *PPP1R26*). The SNP for the DRP of FEED on BTA23 depicted one putative candidate gene (*SLC25A27*).

Table 12: Potential candidate genes, within < 50 kb vicinity, related to the identified SNPs (detected via *fastBAT*) associated with animal behaviour traits.

Trait	Method	BTA[1]	SNP	Candidate gene	Molecular function[2]	Biological process	Start of Gene
RUM	DRP	21	BTB-00811262	*BTBD1*	ubiquitin protein ligase binding	Neurogenesis[2], regulation of proteolysis[2], proteasome-mediated ubiquitin-dependent protein catabolic process[2], key protein of balance between adipogenesis and myogenesis[3], expressed particularly in skeletal muscle[3]	25467798
	DRP	27	ARS-BFGL-NGS-67948	*THAP1*	RNA polymerase II proximal promoter sequence-specific DNA binding, nucleic acid binding, DNA binding, protein binding, zinc ion binding, protein homodimerization activity, metal ion binding	negative regulation of transcription by RNA polymerase II[2], endothelial cell proliferation[2], transcription, DNA-templated[2], regulation of mitotic cell cycle[2]	37234389
	DRP	27	ARS-BFGL-NGS-67948	*RNF170*	Metal ion binding, transferase activity, ubiquitin protein ligase activity	Protein ubiquitination[2]	37246976
	DRP, MEAN	29	ARS-BFGL-NGS-118751	*RPS6KB2*	nucleotide binding, protein kinase activity, ATP binding, transferase activity, transferring phosphorus-containing groups	Protein phosphorylation[2], signal transduction[2], Protein kinase B signalling[2], positive regulation to translational initiation[2]	45965769
	DRP, MEAN	29	ARS-BFGL-NGS-118751	*PTPRCAP*	-	defence response[4]	45972077
	DRP, MEAN	29	ARS-BFGL-NGS-118751	*CORO1B*	protein binding, actin filament binding	actin cytoskeleton organization[2], actin filament branching[4], cellular response to platelet-derived growth factor stimulus[4], endothelial cell chemotaxis[4], negative regulation of smooth muscle cell chemotaxis[4], wound healing[4]	45974636
	DRP, MEAN	29	ARS-BFGL-NGS-118751	*GPR152*	G-protein coupled receptor activity	G-protein coupled receptor activity	45985247
	DRP, MEAN	29	ARS-BFGL-NGS-118751	*CABP4*	calcium ion binding, metal ion binding	visual perception[2], phototransduction[2], photoreceptor cell morphogenesis[2], retinal cone cell development [2], retinal bipolar neuron differentiation [2]	45988625
	DRP, MEAN	29	ARS-BFGL-NGS-118751	*TMEM134*	-	-	45997538
	DRP, MEAN	29	ARS-BFGL-NGS-118751	*AIP*	transcription cofactor activity, protein binding, GAF domain binding, unfolded protein binding	protein folding[2], protein targeting to mitochondrion [2], xenobiotic metabolic process [2], protein maturation by protein folding [2]	46012079
	DRP, MEAN	29	ARS-BFGL-NGS-118751	*PITPNM1*	metal ion binding	transport[2]	46018812
	DRP	29	ARS-BFGL-NGS-118384	*ENSBTAG00000000776*	G-protein coupled receptor activity	G-protein coupled receptor signalling pathway[2]	48988149
	DRP	29	ARS-BFGL-NGS-61470	*MRGPRG*	G-protein coupled receptor activity	G-protein coupled receptor signalling pathway[2]	49026020
ACT	MEAN	19	Hapmap48998-BTA-104140	*PPM1E*	catalytic activity, phosphoprotein phosphatase activity, protein serine/threonine phosphatase activity, hydrolase activity, cation binding, metal ion binding	negative regulation of protein kinase activity[2], protein dephosphorylation[2], cellular response to drug[2], peptidyl-threonine dephosphorylation[2], positive regulation of stress fibre assembly[2]	10143539

FEED	DRP	11	ARS-BFGL-NGS-27581	*STXBP1*	protein binding, syntaxin binding	platelet degranulation[2], vesicle docking involved in exocytosis[2], neurotransmitter secretion[2], neuromuscular synaptic transmission[4], axon target recognition[4], negative regulation of neuron apoptotic process[4]	98325785
	DRP	11	ARS-BFGL-NGS-27581	*CFAP157*	microtubule binding	sperm axoneme assembly[4]	98400784
	DRP	11	ARS-BFGL-NGS-27581	*PTRH1*	aminoacyl-tRNA hydrolase activity	-	98406893
	DRP	11	ARS-BFGL-BAC-15613	*TOR2A*	nucleotide binding, protein binding, ATP binding, nucleoside-triphosphatase activity	chaperone mediated protein folding requiring cofactor[2], protein homooligomerization[2]	98425082
	DRP	11	ARS-BFGL-NGS-13735	*LCN8*	small molecule binding[4]	response to hormone[2]	106391056
	DRP	11	ARS-BFGL-NGS-13735	*LCN15*	transporter activity, small molecule binding	lipid metabolic process[2], transport[2]	106397537
	DRP	11	ARS-BFGL-NGS-21943	*PPP1R26*	protein phosphatase inhibitor activity, protein binding, phosphatase binding	negative regulation of phosphatase activity[2]	106462138
	DRP	23	ARS-BFGL-NGS-80066	*SLC25A27*	Transmembrane transporter activity[4]	Mitochondrial transport[4], regulation of mitochondrial membrane potential[4], response to cold[4], long chain fatty acids uptake gene (Jiang et al., 2009)	19860795

[1]Gene ID (Chromosomal location); Functions derived from Ensembl[2], NCBI[3], UNIPROT[4]; BTA = *Bos taurus* chromosome

Discussion

Characterization of genetic diversity via principal component analysis and WIDDE breed assignment

The two to three clusters, resulting from the PCA, reflected the geographical origin of the European cattle breeds. Figure 14a clearly differentiated between the Holstein lines from northern Europe (Germany, Poland) and the Simmental and Brown Swiss breeds from the south of Europe (Flori et al., 2009), implying that alleles were not common in all breeds. It has to be kept in mind however, that the PCA does not necessarily include geographical information, as the optimization criterion lies within the maximization of genetic variance (Gautier et al., 2011). Gautier et al. (2011) argued, that spatial structuring will not be accounted for, if it is not associated with the most pronounced differentiation. This can be seen with the close ascribing of Sl_HF and DE_HF in one cluster (Figure 14a, b, c), due to their high genetic relationships, in spite of their geographic distance. In this regard, Wiener et al. (2004) reported relationships, based on 30 microsatellites, between breeds, that do not primarily cluster according to geographical origin. They declared, that Scottish breeds (e.g. Highland, Galloway) were not closely related with other breeds from the British Isles (e.g. Dexter, Hereford), regardless of their close geographic distribution. On the contrary, they found surprisingly close genetic connections between the British Friesian, imported from continental breeds and the Scottish Ayrshire. Wiener et al. (2004) justified this relationship with crossbreeding of Ayrshire and Dutch-Flemish cattle for productivity improvement during the 18th century, while Friesians were imported from the same area in the 19th century.

The second distinct cluster represented cattle breeds from the south of Europe (Switzerland), due to the similar genetics of CH_OBS, PL_BS, CH_Si and Sl_Si. Although, in this case geographic effects certainly have contributed to the cluster formation, different breeding goals within each breed were expected to intensify the differentiation as well. As Simmental and CH_OBS are dual-purpose breeds, they are expected to differ from high milk yielding Holstein lines (Chen et al., 2016). Figure 14b and 14c differentiated between the breeds Simmental, Holstein Friesian and Brown Swiss, underlining the footprints of artificial selection more clearly. Although the DE_DN and DE_DSN are considered dual-purpose breeds, they constituted the ancestors

of Holstein Friesian cattle, explaining their close affinities. As a corollary, the PCA recapitulated the geographic origins and endogamous breeding units of Holstein, Brown Swiss and Simmental cattle, emphasizing a 'genetic isolation' (Figure 14b) by the absence of admixture.

The results of the breed assignment were most relevant, in order to comprehend the patterns of genetic diversity and the historical evolutionary processes, helping to uncover the composition of bovine populations. All breeds depicted at least 57.83 % of genetic relations to European cattle breeds, such as Holstein, Hereford, French Red Pied Lowland, Norwegian Red, Red Angus, Bretonne Black Pied, Parthenais, Tarine, Abondance, Charolais, Montbeliarde and French Brown Swiss, affirming their European line of descent. On the other side, exotic ancestral proportions, such as Sheko, Zebu Bororo, Gir or Arabic Zebu were identified within the European genotypes. Despite the fact, that cattle are ascribed to two major geographic types, taurine (humpless- European, African, Asian) and indicine (humped- South Asian, East African), > 250,000 years ago they diverged from common ancestors (Gibbs et al., 2009). Gibbs et al. (2009) confirmed the persistency of the same haplotypes among taurine and within indicine breeds, while the same haplotypes between those two groups were scarce. Ancient genetic ties to a common ancestor as well as interbreeding events explain a relationship of up to 7.09 % between Sheko and Holstein (DE_DSN, DE_DN, DE_HF, PL_HF) and Simmental (Sl_Si) lines. Furthermore, Gibbs at al. (2009) corroborated the descent of European breeds from indicine and taurine crosses, such as Beefmaster, Santa Gertrudis and Sheko. In the present study, low genetic proportions of these taurine crosses were identified within the European genotypes as well.

Payne and Hodges et al. (1997) illustrated, how breeders spread from the Fertile Crescent towards North-West Europe, following two migration routes. One route led to the north along the Balkans' rivers (Danubian route) towards Germany and the Netherlands, while the second route (Mediterranean route) led through western Europe across the Mediterranean Sea towards Italy, Spain and France. During these migration waves, potential interbreeding between wild European aurochs and already domesticated stocks explain the displayed footprints of exotic breeds within the European bovine genome (Gautier et al., 2011). These findings also confirmed the known recent shared ancestry of Holstein and Norwegian Red (Gibbs et al., 2009). In all Holstein lines (DE_DSN,

DE_DN, DE_HF, PL_HF) genetic relations of Norwegian red cattle were detected. Additionally, Gautier et al. (2011) affirmed the Northern European origin of Angus, Red Angus, French and American Holstein, French Red Pied Lowland and Norwegian Red cattle, via Reynolds genetic distances (computed via allele frequencies at 44,706 SNPs), accounting for the depicted genetic kinships in Figure 15. Close genetic proximities between French Red Pied Lowland to Holstein can be explained by the Red Pied Lowland's recent derivation from red Holstein and Meuse-Rhin-Yssel breeds from Germany (Gautier et al., 2011). Relatively high average genetic relationships (8.87-12.39 %) between Hereford and Simmental and Brown Swiss and Holstein in the current study concur with the results of Gautier et al. (2011), where Hereford, Holstein and Brown Swiss were ascribed to one major cluster. Gamarra et al. (2017) explained the historical genetic influence of Hereford on Holstein, Simmental and Brown Swiss by interbreeding events, that occurred before the establishment of the Hereford herd in 1846 (Heath-Agnew, 1983).

As exemplified in the present study, PCA as well as the breed assignment supported the European origin of these breeds (HF, DSN, DN, Si, BS, OBS) and implied distinct geographically variable selective forces (influenced by economic purposes). These evolutionary formative events resulted into different genetic variants in cattle breeds of disparate regions. Moreover, they influenced the differentiation of allele frequencies among populations and the relation between phenotypes and genotypes. Furthermore, it was affirmed that, based on allele-sharing distances between individuals, the extent of ancestry can be predicted rather accurately without pedigree information. Hence, the WIDDE breed assignment can be of great use for the management of endangered bovine populations, whenever pedigree information is missing.

Genetic parameters of sensor traits

Estimating genetic parameters via PGMIX as well as solely on pedigree basis did not significantly affect the outcome of results, ensuring the results' reliability of both methodologies. Basic dairy cattle habits (e.g. sleeping, feeding) underlay moderate genetic control, while RUM, ACT, ET, I and IC resulted into minor heritabilities, which will be more difficult to select for. Index and IC seem to be more of a management tool, that can be applied on farm, in order to reach conclusive insights about an animal's

welfare status but fail to function as indicator traits. Especially the heritability of FEED behaviour concurs well with results from literature. Løvendahl and Munksgaard (2016) derived a moderate heritability of 0.20 in Holstein dairy cattle, while Robinson and Oddy (2004) reported a heritability of 0.36 for time spent feeding in feedlot-finished beef cattle. However, the question remains, of how feeding behaviour ought to be evaluated from a breeder's point of view. Whether long feeding times represent a desirable breeding goal, as opposed to shorter feeding times, one always has to bear in mind the trade-off between feeding and rumination time (Løvendahl and Munksgaard, 2016). Therewith, when breeding for increased milk production in dairy cattle, physiological coping mechanisms, reflected by reduced feeding and resting times of cows, in order to uphold their production level, should be monitored. Especially high yielding cows might compensate for shorter feeding periods by faster food intake or increased rumination efficiency, in order to satisfy their energy requirements. This aspect has been addressed by Løvendahl and Munksgaard (2016), who emphasized the effects of time constraints on dairy cow behaviour.

The estimated heritability for RUM does not comply with the results from literature. Byskov et al. (2017) reported heritabilities of rumination time (microphone-based rumination monitoring) ranging between 0.14-0.44 in primiparous Holstein herds. A reason for the low heritability in the current study may be the high rest variance (29.59) with a comparably low additive genetic variance (0.84-1.00). Moreover, negative individual level correlations were found between rumination time and DMI (r_g= -0.24 to 0.09), and between rumination time and residual feed intake (r_g= -0.34 to -0.03) (Byskov et al., 2017). Then again, other findings in literature described positive relationships between rumination time and intake of forage natural detergent fibre (NDF) (Nørgaard et al., 2010), physical effective fibre (Mertens, 1997), or long-particle alfalfa silage (Krause et al., 2002). Byskov et al. (2017) suspected weak genetic associations between high feed efficiency and increased rumination time. Their line of argument assumed, that high rumination time implied a thorough mastication of feed particles, which facilitates further breakdown of the feed, leading to increased utilization. Thus, high daily RUM percentage could still be a useful selection criterion and function as an indicator trait for feed efficiency. Corresponding to this hypothesis are findings of Gregorini et al. (2015). They observed longer rumination periods in dairy cows with high feed efficiency, compared to cows with low feed efficiency. Overall, RUM behaviour in cattle can be considered as a

functional trait with economic impact on feed costs. As feed costs make up half of the operating costs of European dairy herds (European Commission, 2015), selecting for high feed efficiency in dairy cows is crucial for profitable milk production (Veerkamp, 1998).

The low heritability estimates of ET implied some genetic control of temperature regulation mechanisms, next to the strong effects of environment and production level (Dikmen et al., 2012). Findings from literature revealed heritabilities for rectal temperature to be of moderate strength (0.15-0.17) (Dikmen et al., 2012; Seath, 1947), which do not concur with the range of the current estimations. One reason for the discrepancy between heritabilities could be the difference of measurement procedure (ear surface vs. rectal temperature). Additionally, the surface ET trait in this study has shown to be greatly affected by the environmental temperature and may differ distinctly from rectal and core body temperature. In this regard, the correlation of surface ET and core body temperature should be evaluated, in order to assess, whether ET reflects temperature regulation of dairy cattle in the same way as core body temperature or rectal temperature do. A moderate heritability of body temperature is considered a particularly interesting functional trait in breeding dairy cattle in tropical and subtropical regions for heat tolerance. Olson et al. (2012) generated evidence, supporting the existence of a major gene, that is responsible for producing a very short, sleek hair coat, which allows the cow to maintain a lower rectal temperature. This gene was predominantly found in Senepol cattle and criollo breeds from Central and South America and Carora cows from Venezuela. Even Carora x Holstein F1 crossbred cows showed improved thermotolerance due to inherited slick-haired coats.

Heritabilities for daily ACT (0.06-0.08) confirmed estimates from literature, based on accelerometer recordings of activity monitoring (0.03-0.12) (Ismael et al., 2015; Schöpke and Weigel, 2014). However, the implementation of different measuring devices (accelerometer vs. sensor), the recording lengths and number of animals differed between studies. Schöpke and Weigel (2014) considered a period of > 100 days of accelerometer measurements of 1,171 HF cows and focused on the detection of oestrus, which has not been the case in this study. Nonetheless, their results on genetic parameters support the current findings. They concluded, that normal everyday cattle activity needs to be considered a different trait than the increased activity, which is expressed during oestrus.

This was reflected in slightly different heritability estimates of activity during non-oestrus periods (0.03-0.05) and activity during oestrus (0.12) (Schöpke and Weigel, 2014).

The moderate genetic parameters of NA (0.15-0.17) do not coincide with the heritability estimate of dairy cattle lying/sleeping behaviour (0.01) of Løvendahl and Munksgaard (2016). They suspected differences in milk merit to be the driving force behind lying behaviour and eventual trade-offs between lying and eating time. However, the significantly associated SNP markers of NA present compelling reasons, as do the estimated heritabilities, that NA is under some genetic control.

SNP-based heritabilities

In future studies, heritabilities of sensor traits should be estimated based on SNPs (h^2_{SNP}). Yang et al. (2017) suspected potential biases in pedigree based heritabilities (h^2), due to confounding factors, such as common environmental effects shared between close relatives. Moreover, h^2_{SNP} is unlikely to be contaminated with contributions from non-additive genetic effects, as the correlation between additive and non-additive genetic relationships is small compared to h^2. Speed et al. (2012) claimed, that h^2_{SNP} from genotyped unrelated individuals offers advantages over the pedigree-based approach. Accordingly, dense genotype data allows the exploitation of small differences in the proportions of the genome, that is shared among unrelated individuals. These short genomic regions, inherited from a remote common ancestor, generate LD. Consequently, h^2_{SNP} based on unrelated individuals relate only to causal-variant heritability tagged by SNPs (Speed et al., 2012; Yang et al., 2010). Another aspect is the application of multiple trait analyses based on SNPs, where genetic correlations between complex traits, measured on different samples, can be estimated. This is particularly useful when estimating genetic correlations between multiple diseases, which are often unlikely to co-segregate in sufficiently large pedigrees. Such a case would make it impossible to apply the traditional pedigree-based approach. Hence, estimating a genetic correlation, based on h^2_{SNP}, between any two diseases, using independent case-control data, is possible (Visscher et al., 2014).

Generally, h^2_{SNP} remain smaller than pedigree-based h^2. Yang et al. (2017) reasoned, that not all causal variants, especially those with low frequency, are perfectly tagged by SNPs

used in GWAS. Most of the heritability is not 'missing' but cannot be detected, because the individual effects are too small, to pass stringent significance tests. They proved, that the remaining estimated heritability was due to incomplete LD between causal variants and genotyped SNPs. This is aggravated by causal variants, that have a lower MAF than the genotyped SNPs (Yang et al., 2010). In other words, SNPs do not fully explain estimated heritabilities, because the SNPs on the arrays are not in complete LD with the causal variants. The potential of SNPs to explain phenotypic variation, due to causal variants, depends on the LD between all causal variants and all SNPs (Yang et al., 2010). In order to avoid large biases due to LD, overestimating regions of strong LD and underestimating regions of low LD, different methodologies are suggested. Yang et al. (2010) recommended a transformation, including uniformly scaling the usual SNP-based kinship coefficients, while Speed et al. (2012) suggested a weighting of SNPs, depending on how well they are tagged by their neighbours.

In order to estimate h^2_{SNP} however, rather large sample sizes are required, as the information comes from very small coefficients of genetic relationship for pairs of individuals (n > 10,000) (Yang et al., 2017). Thus, the aforementioned small samples size of the current study presents an obstacle in this regard.

Multi-breed GWAS

The identification of the *DGAT1* gene on BTA14 favoured the a priori conclusion that, despite the small sample size, the multi-breed GWAS was able to identify putative causative variants and candidate genes. The gene-based test and *fastBAT* procedure led to the detection of 7 SNPs, which were in close proximity (50 kb) to 22 putative candidate genes, that may affect cattle behaviour such as RUM, ACT and FEED. With an FDR of 20 %, the detection power was increased, compared to applying the stricter BF correction. However, the risk of detecting false positive SNPs was raised as well.

This is the first study, where large data volume of repeated measurements of dual-purpose cows from pasture-systems was condensed to one value per cow, using two different approaches (MEAN, DRP). Hence, it is complicated to assess the value of each approach and to decide, which methodology will proof best in the future. Possibly, there is an even better procedure for processing this kind of data in an appropriate manner for GCTA. However, it can be assumed, that the DRP better captures and sums up the daily behaviour

expression in one value than the MEAN does. The reason behind this hypothesis is, that the statistical model, for estimating the breeding values of sensor traits, already corrected for fixed and random effects (breed-farm, year-month of measurement, age of cow, permanent environment, additive genetic effect), which influenced bovine behaviour. In this regard, Speed et al. (2012) explained, the estimation of random effects ('breeding values') is of primary importance, because these values reflect a 'true' phenotype adjusted for environmental effects or measurement error. On the other hand, when using the MEAN in GCTA, the data was only corrected for the 'breed-farm' effect. Consequently, it is most likely, that the MEAN of sensor behaviour traits, does not correctly reflect the genetic variance of bovine behaviour, due to confounding environmental effects. Thus, a more precise picture of the true phenotype might be gained via DRP, by pre-correcting for fixed and random effects in the DMU model, when calculating the EBVs. The multi-breed GWAS results supported this assumption, as only one significant SNP was detected via the MEAN approach and seven significant SNPs were discovered using DRP.

It is the polygenic nature of most complex traits and diseases, that the effect size of individual variants is too small, in order to be detected, despite large sample sizes (Bakshi et al., 2016). Ample evidence even suggested, that complex trait-associated genetic variants, discovered via GWAS, are enriched in genic regions (Yang et al, 2011a; Schork et al., 2013) and in many cases, there are multiple associated variants at a single locus (Yang et al., 2012; Wood et al., 2014). Due to this phenomenon, Bakshi et al. (2016) reasoned, that for the discovery of complex trait genes, it is more powerful to test the accumulated effect of a set of SNPs, applying set-based association. According to this hypothesis, the a priori conclusion was formulated, that the issue of a 'polygenic' architecture would also affect bovine behaviour traits, marking the gene-based test in GCTA (*fastBAT* option) the best methodology to pursue. Applying *fastBAT*, Bakshi et al. (2016) discovered six novel gene loci for height, two for body mass index and three for schizophrenia. They argued, that the detection power of *fastBAT* was increased, due to multiple small independent association signals at these loci and $p_{fastBAT}$ was smaller than p_{GWAS}. Nonetheless, if there is only one causal variant at a locus, $p_{fastBAT}$ was expected to be larger than p_{GWAS} of the top associated SNP (Bakshi et al., 2016). The current results have shown, that next to the identified significant SNPs from Table 11, other SNPs were discovered (via gene-based test and *fastBAT*) and associated with potential candidate

genes (Table 12). The only SNP, that was detected in GWAS and in the gene-based test was ARS-BFGL-NGS-80066 (BTA23, FEED). These results support the assumption, that there could be more independent association signals (variants) at certain loci, which are simply not tagged by GWAS, due to their small effect size, but which are discovered via *fastBAT*.

The innovative character and the small sample size of this study, make it difficult to clearly associate the identified candidate genes directly to bovine behaviour, such as RUM, ACT or FEED. However, some of the discovered potential candidate genes were already linked to traits or diseases in cattle, humans, pigs or mice.

Rumination. The putative candidate gene *RPS6KB2* was associated with innate immune response in indigenous and crossbred cattle (Thapakar, Vrindavani) (Chauhan et al., 2015). Moreover, Chen et al. (2011) discovered the gene to be differentially expressed in the liver transcriptome of Angus cattle, that were genetically selected for low and high residual feed intake. Meade et al. (2007) identified the gene to be differently expressed in bovine tuberculosis- infected and control cattle. The real time quantitative PCR confirmed a trend of innate immune gene repression for *RPS6KB2*. Other findings from literature implied an association of *RPS6KB2*, as a TGF-β gene, with pre-implantation embryonic development in cattle (Li et al., 2012).

Another potential candidate gene, which was associated with RUM behaviour was the *PTPRCAP* gene. The gene was suggested to be involved in the defence response in humans (Zerbino et al., 2018) as a key regulator of lymphocyte activation, while Fuka et al. (2011) found it to be repressed by the Leukemia-Specific Fusion Gene *ETV6/RUNX1*.

The putative candidate gene *CaBP4*, coding for a neuronal Ca2+-binding protein, was described to be expressed in photoreceptors in mice, where it is localized to synaptic terminals (Haeseleer et al., 2004). Haeseleer et al. (2004) further concluded, that the phenotype of *Cabp4*−/− mice shared similarities with patients of incomplete congenital stationary night blindness (CSNB2). Generally, *CaBP4* is suggested to play an important role in normal synaptic function (Haeseleer et al., 2004).

A rather interesting association was found between the potential candidate gene *TMEM134* and obesity as well as atherosclerosis in adults (Keustermans et al., 2017). Furthermore, Literature implied, that *TMEM134* was involved in the prototypical inflammatory nuclear factor-κB (NF-κB) signalling pathway (Keustermans et al., 2017).

The modulation of downstream NF-κB signalling was reasoned to be one of the hallmarks of innate immune programming in chronic inflammation (Morris et al., 2014). However, whether the downregulation of the *TMEM134* has a distinct effect on cattle remains to be elucidated. The identified *PITPNM1* gene was associated with retinal degeneration and hypopyon in human; amongst its related pathways were metabolism and glycerophospholipid biosynthesis (Stelzer et al., 2016).

Activity. The potential *PPM1E* candidate gene was linked with ACT behaviour in dual-purpose cattle. Inter alia, the dephosphorylation gene was associated with feeding behaviour in Danish Duroc boars (Do et al., 2013). Do et al. (2013) assumed, that *PPM1E* is mediated by 5'AMP-activated protein kinase (AMPK), which is well documented to play key roles in controlling energy balance. It was assumed, that AMPK is involved in hypothalamic glucose and nutrient sensing. There is some basis for argumentation, that *PPM1E* can be related to activity and feeding behaviour. The previous study in chapter 3 revealed an indistinct relationship between ACT behaviour and BCS. Dual-purpose cows, that expressed low levels of daily ACT showed significantly higher BCS, as cows, that were more active. It was argued, that cows, which spend more time feeding, ruminating and being active, had a generally lower BCS, due to higher milk yield. On the other hand, cows with increased BCS, but reduced activity, feeding and rumination behaviour had lower milk yields. This trend was confirmed by Roche et al. (2009), who concluded, that body condition loss correlated positively with cow activity and peak milk yield. As a corollary, it can be stated that, in spite of an association with *PPM1E* and pig feeding behaviour, bovine activity may also be associated with *PPM1E*, as phenotypic relations between feeding and activity were assumed.

A straighter forward aspect of the *PPM1E* gene was depicted in the study of Detmer et al. (2008). They demonstrated hindlimb gait defects, due to decreased hindlimb muscle mass and reduced number of motor axons, in transgenic mice. Notably, the transgene was integrated into chromosome 11 between the *Rad51c* and *PPM1E* genes (Detmer et al., 2008). Due to these findings, it can be assumed, because of the *PPM1E*'s proximity to the transgene, that *PPM1E* is involved in the locomotor system and the distribution of axonal mitochondria in motor neurons as well.

Feeding. The potential *LCN15* candidate gene was associated with FEED in dual-purpose cattle. Notably, existing studies indicated the gene's involvement in transport of glucose and other sugars, bile salts and organic acids, metal ions and amine compounds as well as transport of vitamins and nucleosides (Stelzer et al., 2016). As a member of the lipocalin gene family, of which *LCN2* is assumed to be involved in obesity and diabetes in human (Boztepe and Gulec, 2018), it can be hypothesized, that *LCN15* plays a role in feeding behaviour in dual-purpose cattle. Boztepe and Gulec (2018) have shown, that elevated glucose dosages distinctly induced levels of *LCN15* transcription. Furthermore, it was proposed, that *LCN15* physiologically interacted with high glucose levels in enterocytes. Hence, the intestinal absorption of glucose may, among other genes, be controlled by *LCN15* (Boztepe and Gulec, 2018).
The potential candidate gene *SLC25A27* was also associated with FEED. Jiang et al. (2009) identified the gene to be part of a novel genetic network, associated with economically important traits in Wagyu x Limousin cattle (Jiang et al., 2009).

The multi-breed GWAS study revealed various interesting aspects and novel approaches, which is why further research in this field is strongly recommended. Especially, because of the complexity of traits, it would be desirable to increase the sample size as well as the SNP density to imputed full-sequence genotypes, as the SNP coverage was insufficient for some regions within the genome. McClure et al. (2012) also mentioned different SNP densities, which possibly affected the results. Moreover, Sanchez et al. (2017) described, that in their multi-breed GWAS, significant differences between markers (50 K, HD SNP, or sequence) led to variations of peaks within the Manhattan plots. While there were several peaks identified for whole-genome sequences, only one peak was observed for 50 K SNP density.
Although the reliable detection of the *DGAT1* gene assured the workability of the multi-breed GWAS approach on a small sample size, Raven et al. (2014) clearly stressed the effect of sample size on detection power. They combined data of Holstein bulls and cows, instead of analysing them separately. McClure et al. (2012) reached similar conclusions when conducting within and across breed GWAS on beef breeds (Angus, Hereford, Simmental, Charolais, Limousin). They reasoned, that the within breed analyses were underpowered to detect segregating loci, due to smaller sample sizes.

When increasing the sample size, the conduction of a within breed GWAS is recommended. This way, significantly detected SNPs within single breeds can be compared and it becomes clearer, whether there are differences in proportion of significant variants between breeds. Another aspect is the detection of private alleles or breed-specific QTLs, which would require within-breed GWAS. Also, the number of significant variants, detected per trait, may increase when using within-breed analyses, as Sanchez et al. (2017) reported for their within-breed analyses.

Conclusion

This study provided evidence of a clear clustering of the genetic relatedness between European dual-purpose cattle. The heritability estimates for behaviour traits were similar for pedigree-based and single-step GBLUP. As expected, the genetic parameters of the electronically recorded bovine behaviour traits, such as rumination, feeding, activity, high activity, sleeping and ear temperature remained within a rather low range and showed acceptable standard errors. In spite of the small sample size, reliable heritability estimates were generated, due to multiple repeated measurements per animal. A combination of sensor traits into one indicator trait, such as index and index class, did not reveal sufficient heritability and should rather be used as a reference value for management decisions.

Despite the low sample size, it is worth noting, that the multi-breed GWAS reliably detected the region within the genome, containing genes, known to be related to fat production, such as *DGAT1*. The limited number of individuals analysed and the complex nature of behaviour traits examined, which are influenced by environmental factors, may have a negative influence on the power of this analysis. Moreover, the SNP coverage was not evenly distributed across the 29 autosomes. However, significant SNPs for rumination, activity and feeding were detected. Applying the gene-based test and the *fastBAT* procedure, allowed an identification of plausible candidate genes, which could harbour mutations, affecting bovine behaviour. In the future, using sequence data rather than SNP array genotypes combined with a multi-breed analysis and increased records of phenotypes and genotypes, could result into direct identification of causative mutations in natural cattle behaviour. A discovery of potential causative mutations may have immediate applications for improvements in selecting dual-purpose cows, that are better adjusted for current and future production systems in terms of behaviour. Continuing research in the field of multi-breed GWAS, with regard to behaviour traits, seems most

promising. Only a combination between genetic, genotypic and phenotypic measures of complex traits will complement the physiological characterisation of cattle. This aspect is particularly important, in the case of endangered dual-purpose breeds, as it can contribute to conservation and genetic resource management decisions.

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CHAPTER 5

Variance Heterogeneity and Genotype by Environment Interactions in Native Black and White Dual-Purpose Cattle for Different Herd Allocation Schemes

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Introduction

Daughter records of influential artificial insemination (AI) sires are available in different environments, allowing i) estimations and comparisons of genetic correlations between same traits across country borders and ii) ranking of the same bulls on different national scales according to environmental classifications (Hammami et al., 2009). For bull comparisons across country borders, Schaeffer (1994) developed the multiple-trait across country evaluation (MACE) methodology. Instead of country borders, Weigel and Rekaya (2000) suggested a 'borderless clustering' approach for genetic evaluation of sires, where herds were allocated into different clusters, based on their management practices, climatic conditions, or genetic herd architectures. The idea behind this approach being, that herds located in different countries may be more similar regarding management, climate and genetic compositions, than herds from the same country. Against this background, various studies considered the aspect of genotype by environment interactions (GxE), defining different environments (Weigel and Rekaya, 2000), feeding systems (Kolver et al., 2002), herd protein production levels (Kolmodin et al., 2002) or management and housing characteristics (Fatehi et al., 2003) within and across countries. Instead of a multiple-trait animal model (MTAM) application, Kolmodin et al. (2002) studied phenotypic and genetic variations for protein yield on a continuous herd scale via reaction norm models. In this regard, most of the GxE studies were conducted in large dairy cattle populations, especially in Holstein Friesian (HF).

Byrant et al. (2006) studied productivity for a range of different feeding levels. "Environmental sensitivity" was more obvious in high yielding North American HF, compared to New Zealand HF or Jerseys. In consequence, Byrant et al. (2006) described North American HF strains as specialists (high production output in superior feeding level environments), and New Zealand HF and Jersey breeds as generalists, tolerating environmental changes. Such a genetic line characterisation supports the hypothesis of generalists evolving in heterogeneous, and of specialists (e.g. HF) in homogeneous environments (Byrant et al., 2006). Kolmodin et al. (2002) confirmed this hypothesis. They detected significant variations between sires regarding daughter sensitivity in different farm environments. Sires with a high genetic merit for production traits reacted more sensitive to changes in herd environments than sires with low a genetic merit.

Changes on the European milk market with decreasing prices and increasing fodder costs force dairy farmers to switch from conventional to alternative dairy production systems, i.e. changing from high-input indoor to low-input pasture-based production. However, there are certain requirements for dairy cows kept in grazing systems, regarding metabolic stability, robustness and fertility (Dillon et al., 2003). The shift in feeding and thus, reduced energy intake, especially affects high producing breeds having been selected for indoor systems for decades, such as HF (Dillon et al., 2003).

Due to their long process of adaptation to grassland or ecological systems, it can be hypothesized, that so-called indigenous dual-purpose cattle are more robust to challenges in harsh environments, such as low fodder quality, heat stress or disease infections. In such context, the definition of robustness also implies quite homogeneous genetic values and genetic (co)variance components with environmental alterations (König, 2017). The black and white dual-purpose cattle (DSN) have the ability to efficiently convert pasture into milk, without feeding concentrates in small-scale grassland or organic family farms (Jürgens et al., 2015; Gassan, 2017). Belonging to the lowland breeds, DSN cattle originated from the coastal grassland regions of northern Germany and the Netherlands. Black and white cattle populations from the Netherlands and Poland are descendants from common German founders, and considered as a robust breed for similar grassland production systems (Nauta et al., 2006; Gassan, 2017; Biedermann, 2003). Hence, in order to provide optimal genetic material for specific European environments, a borderless estimation of breeding values might be an alternative for strict national genetic evaluations for DSN.

Nevertheless, the quality of across country genetic evaluations or borderless clustering approaches via MTAM strongly depends on genetic connectedness among clusters or herd strata. In such context, König et al. (2002) hypothesized impact on genetic relationships on genetic (co)variance component estimates. Clément et al. (2001) stated, that also an AI program might contribute to poor genetic connectedness, i.e., when only operating within specific regions - as being the case for endangered breeds. In regional breeds of dairy goats, Analla et al. (1995) pointed out, that poor genetic connectedness and sparse pedigree information were main causes for biased breeding values with low prediction accuracies.

So far, there is only limited research addressing the impact of heterogenic environmental effects and of genetic connectedness on genetic (co)variance estimates in endangered native dual-purpose cattle breeds. In consequence, this study aimed at defining environmental and herd descriptors, in order to follow the 'multiple-trait herd cluster' approach (Weigel and Rekaya, 2000) for the DSN breed. Specifically, the objectives of this study were i) to estimate genetic (co)variance components in discrete herd environments via MTAM applications, and ii) to study genetic correlation estimates against the background of genetic connectedness of DSN sub-populations. Results for DSN were compared with genetic parameter estimates from HF and "mixed" breed analyses, using the same herd data base and same strategies for data stratification according to environmental and herd descriptors.

Materials and Methods

DSN herds and traits

The majority of farms was located in the northwest grassland region in East Friesland and around Hannover and Bremen. Three major geographic regions were determined: 1. intensive grazing systems on coastal marshlands, 2. large-scale farms in one region of former East Germany, and 3. small-scale family farms in the middle and the south of Germany. Apart from the eastern German region, farms predominantly reflected pasture-based low-input production systems.

The study included 3,659 DSN and 2,324 HF cows from parities one to three, kept in 46 different herds. Herds always kept DSN cows, but in most cases, herds were 'mixed' herds (Mixed) including both genetic lines (HF and DSN). In calving years 2014 and 2015, herd sizes ranged from 11 to 1,712 cows, with an average of 130 cows per herd. With a focus on the naturally negative energy balance during the early lactation period, traits of interest were test-day records from the first official test-day after calving for a production trait (milk yield: Mkg), an indicator trait for udder health (somatic cell score: SCS) and a trait, reflecting metabolic stability (fat-to-protein ratio: FPR). Cow test-day traits represented the first three lactations from calving years 2010 to 2015. Descriptive statistics for the test-day traits are given in Table 13.

Table 13: Descriptive statistics (standard deviation: SD; minimum: Min; maximum: Max) for milk yield (Mkg), somatic cell score (SCS) and fat-to-protein ratio (FPR) of the first official test-day after calving for the genetic lines: black and white dual-purpose cattle (DSN), Holstein Friesian (HF) and Mixed (HF+DSN).

	Mixed				DSN				HF			
Trait	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max
Mkg	25.01	7.21	2.40	62.40	23.77	6.66	2.40	52.50	26.96	7.61	6.00	62.40
SCS	4.64	1.75	-0.64	11.64	4.58	1.70	-0.64	11.64	4.74	1.81	0.36	11.64
FPR	1.28	0.24	0.46	2.74	1.25	0.22	0.46	2.74	1.33	0.25	0.48	2.55

Herd grouping

The chosen herd descriptors for herd grouping considered classical management factors (average herd size, average herd calving age), production characteristics (average herd milk production level, average herd somatic cell count level), genetic descriptors (average percentage of DSN cows within herds, average percentage of cows with natural service sires within herds), and also strict environmental descriptors (altitude and latitude of farm). Consequently, eight discrete descriptive herd descriptors were defined for herd grouping. Herd grouping according to herd descriptor means considered all cows (i.e., HF and DSN) from the same herd and was done as follows (Table 14): Herd size (H-SIZE; group 1: <250 milking cows; group 2: >250 milking cows), herd calving age (H-CA: group 1: <38 months; group 2: >38 months), herd milk production level from the first test-day (H-MPL; group 1: <30 kg; group 2: >30 kg), herd somatic cell count from the first test-day (H-SCC; group 1: <200,000 cells/ml; group 2: >200,000 cells/ml), herd percentage for DSN cows (H-DSN %; group1: <25 %; group 2: >25 %), percentage of herd with natural service sire (H-NSS; group 1: <30 %; group 2: >30 %), altitude of farm (F-ALTITUDE; group 1: <60 m above sea level; group 2: >60 m above sea level) and latitude of farm (F-LATITUDE; group 1: <52.2°; group 2: >52.2°). An overview of the number of cows within herd groups for all herd descriptors is given in Table 15.

Table 14: Applied herd and geographical descriptors and respective thresholds for the classification of herds into herd group 1 or herd group 2.

Herd / geographical descriptor	Group 1	Group 2
Ø Herd size (H-SIZE)	< 250 cows	> 250 cows
Ø Calving age (H-CA)	< 38 months	> 38 months
Ø Milk production level of herd (H-MPL)	< 30 kg	> 30 kg
Ø Somatic Cell Count of herd (H-SCC)	< 200,000 cells/ml	> 200,000 cells/ml
Ø Percentage of DSN genetics (H-DSN %)	< 25 %	> 25 %
Ø Percentage of natural service sire (H-NSS)	< 30 %	> 30 %
Altitude of farm (F-ALTITUDE)	< 60 m above sea level	> 60 m above sea level
Latitude of farm (F-LATITUDE)	< 52.2°	> 52.2°

Table 15: Number of cows within herd groups 1 or 2 for different herd descriptors and genetic lines (Mixed: DSN + HF, DSN: black and white dual-purpose cattle, HF: Holstein Friesian).

	Mixed		DSN		HF	
Herd descriptor[1]	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2
H-SIZE	6,392	4,448	2,941	3,859	3,451	589
H-CA	5,314	5,526	4,012	2,788	1,302	2,738
H-MPL	5,917	4,923	3,414	3,386	2,503	1,537
H-SCC	5,415	5,425	4,717	2,083	698	3,342
H-DSN %	5,430	5,410	1,581	5,219	3,849	191
H-NSS	5,354	5,486	3,746	3,054	1,608	2,432
F-ALTITUDE	5,949	4,891	2,680	4,120	3,269	771
F-LATITUDE	5,007	5,833	4,091	2,709	916	3,124

[1]Herd descriptor, explained in Table 14

Additionally, herds were clustered considering all eight herd descriptors simultaneously. The FASTCLUS procedure of SAS University Edition was applied, in order to generate disjoint herd clusters based on Euclidean distances (SAS Institute Inc., 2008) for standardized herd descriptors (herd descriptor variables were standardized applying the STANDARD procedure in SAS). In this regard, herds were allocated to three groups (clusters). Defining a larger number of clusters was associated with challenging computation requirements and convergence problems and a limited number of herds and cows per cluster. Descriptive statistics for herd descriptors within the three clusters are presented in Table 16. The clusters mostly varied with regard to farm location. The first cluster included 17 herds and was located in the South (48°67´N - 52°64´N latitude), the second cluster included 28 herds from the North (51°8´N-54°52´N latitude), and the third cluster included only one large-scale dual-purpose cattle herd from the East at 51°66´N. Herds from clusters 1 and 2 were quite similar regarding H-SIZE (92 cows and 96 cows, respectively), H-MPL (24.88 kg and 25.11 kg, respectively), H-SCC (282,996 cells/ml and 270, 869 cells/ml, respectively), H-DSN % (both groups 19 %) and H-NSS (49 % and 58 %, respectively). While clusters 1 and 3 showed similar means for H-CA (39.27 and 37.55 months, respectively) and F-LATITUDE (51°12 and 51°67, respectively), clusters 2 and 3 differed substantially for all herd descriptors.

Table 16: Mean values for herd descriptors for clusters 1, 2 and 3.

Herd descriptor	Cluster 1 (17 farms)	Cluster 2 (28 farms)	Cluster 3 (1 farm)
	Mean	Mean	Mean
H-SIZE	92	96	1,712
H-CA	39.27	43.03	37.55
H-MPL	24.88	25.11	30.00
H-SCC	282,996	270,869	175,140
H-DSN %	19.00	19.00	36.00
H-NSS	49.00	58.00	26.00
F-ALTITUDE	200.47	14.68	84.00
F-LATITUDE	51°12	53°28	51°67

Genetic relationships within and between groups and clusters

Inbreeding coefficients and pedigree based genetic relationships between and within herd descriptor groups and clusters were calculated applying the software package CFC (Sargolzaei et al., 2006). The pedigree considered 74,319 individuals from birth years 1925 until 2013.

Estimation of genetic parameters

All analyses were done separately for all combinations of genetic lines with herd descriptors. For the MTAM applications, same traits were considered as different traits in herd groups 1 and 2. Estimation of genetic (co)variance components was accomplished in bivariate runs, using the software package VCE (Groeneveld et al., 2010). The same cow cannot perform simultaneously in groups 1 and 2. Hence, non-existing residual covariances among traits were assumed. Same analyses were performed i) considering all cows from both genetic lines DSN and HF from the same herd (i.e., Mixed analyses), ii) only considering cows from the DSN line, and iii) only considering cows from the HF line. In matrix notation, the genetic statistical model including the two traits was:

$$\begin{bmatrix} \mathbf{y_1} \\ \mathbf{y_2} \end{bmatrix} = \begin{bmatrix} \mathbf{X_1 b_1} + \mathbf{Z_1 u_1} + \mathbf{W_1 p_1} + \mathbf{e_1} \\ \mathbf{X_2 b_2} + \mathbf{Z_2 u_2} + \mathbf{W_2 p_2} + \mathbf{e_2} \end{bmatrix}$$

where y_1 and y_2 were vectors of test-day records for cows in groups 1 and 2, respectively; b_1 and b_2 were vectors of fixed effects including the genetic line effect in the Mixed analyses, with a combined effect of year and season of the test-day, age of calving as covariable, herd and parity classes in all studies for the two traits; u_1 and u_2 were vectors of additive-genetic animal effect for the two traits; p_1 and p_2 were vectors of permanent

environmental effect for the cows for the two traits (repeated measurements due to trait recording in different parities); and e_1 and e_2 were vectors of random residual effects for both traits. X_1, X_2, Z_1, Z_2, W_1 and W_2 were incidence matrices for b_1, b_2, u_1, u_2, p_1 and p_2, respectively. The variance-covariance structure for the random effects was:

$$\text{var}\begin{bmatrix} u_1 \\ u_2 \\ p_1 \\ p_2 \\ e_1 \\ e_2 \end{bmatrix} = \begin{bmatrix} g_{11}A & g_{12}A & 0 & 0 & 0 & 0 \\ g_{21}A & g_{22}A & 0 & 0 & 0 & 0 \\ 0 & 0 & \sigma^2_{p1}I_{p1} & 0 & 0 & 0 \\ 0 & 0 & 0 & \sigma^2_{p2}I_{p2} & 0 & 0 \\ 0 & 0 & 0 & 0 & r_{11} & 0 \\ 0 & 0 & 0 & 0 & 0 & r_{22} \end{bmatrix}$$

where g_{11} and g_{22} were additive-genetic variances of the two traits; g_{12} and g_{21} were additive-genetic covariances between both traits; A was the additive-genetic relationship matrix, σ^2_{p1} and σ^2_{p2} were the variances for permanent environmental effects for both traits; I_{p_1} and I_{p_2} were identity matrices for the cows with records for both traits; r_{11} and r_{22} were residual variances for both traits.

Results

Variance components and heritabilities within herd groups and clusters

Variance components for additive-genetic, permanent environmental and residual effects as well as corresponding heritabilities stratified by genetic group analyses (Mixed, DSN, HF) are presented in Table 17 for Mkg, in Table 18 for SCS and in Table 19 for FPR. As expected, heritabilities were generally larger for the production trait Mkg compared to the functional traits SCS and FPR. This was observed for all genetic group analyses. Heritabilities for Mkg were in a moderate range from 0.13 to 0.36, but only in the range from 0.02 to 0.22 for FPR, and even lower for SCS ranging from 0.01 to 0.16. In general, additive-genetic variances and heritabilities were larger for all traits in herd groups indicating superior herd management. This was the case for H-SIZE with higher additive-genetic variances and heritabilities in group 2 (= large herds), for H-CA with higher additive-genetic variances and heritabilities in group 1 (= low calving age), for H-MPL with higher additive-genetic variances and heritabilities in group 2 (= high milk yield

production level), and for H-SCC with higher additive-genetic variances and heritabilities in group 1 (= low somatic cell count level).

Table 17: Variance components (ϑ_a^2: additive-genetic variance, ϑ_{pe}^2: permanent environmental variance, ϑ_e^2: residual variance) and heritabilities (h^2) for milk yield from the first test-day after calving within groups (Gr) of herd descriptors, stratified by genetic lines (Mixed: DSN + HF, DSN: German black and white dual-purpose cattle, HF: Holstein Friesian) (standard errors of h^2 for all calculations <0.09).

	Mixed								DSN								HF							
Herd descriptor	ϑ_a^2		ϑ_{pe}^2		ϑ_e^2		h^2		ϑ_a^2		ϑ_{pe}^2		ϑ_e^2		h^2		ϑ_a^2		ϑ_{pe}^2		ϑ_e^2		h^2	
	Gr1	Gr2	Gr1	Gr2	Gr1	Gr2	Gr1	Gr2	Gr1	Gr2	Gr1	Gr2	Gr1	Gr2	Gr1	Gr2	Gr1	Gr2	Gr1	Gr2	Gr1	Gr2	Gr1	Gr2
H-SIZE	4.70	6.61	0.00	4.39	23.21	25.26	0.17	0.18	4.57	6.71	0.00	4.41	18.86	23.65	0.20	0.19	4.57	8.26	0.12	2.58	26.71	34.95	0.15	0.18
H-CA	6.31	4.61	3.73	0.00	26.24	22.16	0.17	0.17	6.78	4.31	4.14	0.00	23.67	18.69	0.20	0.19	7.57	4.11	0.82	0.36	31.67	25.70	0.19	0.14
H-MPL	4.44	7.48	0.00	3.25	21.14	27.73	0.17	0.19	4.71	8.87	0.00	4.12	18.50	23.33	0.20	0.24	4.05	7.97	0.01	0.00	24.62	33.18	0.14	0.19
H-SCC	5.44	5.19	2.58	0.34	24.52	26.02	0.17	0.16	5.96	5.21	2.84	0.00	24.40	19.86	0.18	0.21	6.38	4.92	0.15	0.46	21.22	29.47	0.23	0.14
H-DSN %	4.42	6.36	0.70	1.96	25.82	25.67	0.14	0.19	3.86	6.37	0.00	1.94	18.78	25.80	0.17	0.19	4.62	11.56	0.65	0.00	28.63	20.71	0.14	0.36
H-NSS	5.71	5.21	2.58	0.11	27.16	23.72	0.16	0.18	6.33	5.36	3.92	0.00	24.64	18.88	0.18	0.22	4.91	4.98	1.22	0.00	27.07	29.16	0.15	0.15
F-ALTITUDE	5.01	6.01	0.66	2.60	25.08	25.23	0.16	0.18	5.10	6.88	0.00	2.92	19.48	24.96	0.21	0.20	4.55	5.01	1.22	0.00	29.50	22.28	0.13	0.18
F-LATITUDE	6.27	5.16	2.42	0.37	26.62	23.98	0.18	0.17	7.25	5.05	2.65	0.00	25.40	18.81	0.21	0.21	5.59	4.67	0.00	0.93	28.21	28.26	0.17	0.14

Table 18: Variance components (ϑ_a^2: additive-genetic variance, ϑ_{pe}^2: permanent environmental variance, ϑ_e^2: residual variance) and heritabilities (h^2) for somatic cell score from the first test-day after calving within groups (Gr) of descriptive herd descriptors stratified by genetic lines (Mixed: DSN + HF, DSN: German black and white dual-purpose cattle, HF: Holstein Friesian) (standard errors of h^2 for all calculations <0.09).

	Mixed								DSN								HF							
Herd descriptor	ϑ_a^2		ϑ_{pe}^2		ϑ_e^2		h^2		ϑ_a^2		ϑ_{pe}^2		ϑ_e^2		h^2		ϑ_a^2		ϑ_{pe}^2		ϑ_e^2		h^2	
	Gr1	Gr2	Gr1	Gr2	Gr1	Gr2	Gr1	Gr2	Gr1	Gr2	Gr1	Gr2	Gr1	Gr2	Gr1	Gr2	Gr1	Gr2	Gr1	Gr2	Gr1	Gr2	Gr1	Gr2
H-SIZE	0.18	0.35	0.12	0.21	2.91	2.42	0.06	0.12	0.24	0.30	0.15	0.24	2.76	2.37	0.08	0.10	0.14	0.51	0.09	0.00	3.02	2.62	0.04	0.16
H-CA	0.29	0.19	0.24	0.09	2.47	2.94	0.10	0.06	0.28	0.26	0.27	0.12	2.36	2.81	0.10	0.08	0.49	0.11	0.00	0.10	2.71	3.06	0.15	0.03
H-MPL	0.16	0.29	0.21	0.17	2.84	2.53	0.05	0.10	0.18	0.29	0.28	0.19	2.65	2.45	0.06	0.10	0.10	0.40	0.16	0.00	3.10	2.65	0.03	0.13
H-SCC	0.30	0.18	0.19	0.14	2.35	3.07	0.10	0.05	0.29	0.21	0.22	0.25	2.35	2.98	0.10	0.06	0.35	0.20	0.00	0.09	2.28	3.10	0.13	0.06
H-DSN %	0.19	0.34	0.08	0.21	2.92	2.48	0.06	0.11	0.21	0.35	0.11	0.18	2.79	2.49	0.07	0.12	0.21	0.02	0.04	0.95	2.98	2.35	0.06	0.01
H-NSS	0.19	0.27	0.19	0.15	2.59	2.83	0.06	0.08	0.25	0.24	0.18	0.27	2.51	2.62	0.09	0.08	0.10	0.31	0.18	0.00	2.71	3.10	0.03	0.09
F-ALTITUDE	0.18	0.29	0.21	0.10	2.77	2.64	0.06	0.10	0.17	0.28	0.35	0.14	2.60	2.54	0.06	0.09	0.24	0.27	0.06	0.06	2.91	3.09	0.07	0.08
F-LATITUDE	0.30	0.16	0.08	0.24	2.71	2.70	0.10	0.05	0.29	0.16	0.12	0.37	2.55	2.59	0.10	0.05	0.30	0.20	0.00	0.11	3.35	2.81	0.08	0.06

Table 19: Variance components (ϑ_a^2: additive-genetic variance, ϑ_{pe}^2: permanent environmental variance, ϑ_e^2: residual variance) and heritabilities (h^2) for fat-to-protein ratio from the first test-day after calving within groups (Gr) of descriptive herd descriptors stratified by genetic lines (Mixed: DSN + HF, DSN: German black and white dual-purpose cattle, HF: Holstein Friesian) (standard errors of h^2 for all calculations <0.03).

	Mixed								DSN								HF							
Herd descriptor	ϑ_a^2		ϑ_{pe}^2		ϑ_e^2		h^2		ϑ_a^2		ϑ_{pe}^2		ϑ_e^2		h^2		ϑ_a^2		ϑ_{pe}^2		ϑ_e^2		h^2	
	Gr1	Gr2	Gr1	Gr2	Gr1	Gr2	Gr1	Gr2	Gr1	Gr2	Gr1	Gr2	Gr1	Gr2	Gr1	Gr2	Gr1	Gr2	Gr1	Gr2	Gr1	Gr2	Gr1	Gr2
H-SIZE	0.005	0.007	0.002	0.001	0.043	0.035	0.107	0.158	0.006	0.006	0.000	0.001	0.043	0.034	0.129	0.154	0.004	0.009	0.005	0.001	0.041	0.036	0.080	0.194
H-CA	0.007	0.005	0.001	0.002	0.037	0.043	0.168	0.094	0.006	0.007	0.001	0.000	0.034	0.043	0.150	0.135	0.011	0.003	0.000	0.005	0.040	0.041	0.219	0.053
H-MPL	0.005	0.007	0.002	0.000	0.041	0.038	0.106	0.163	0.007	0.006	0.000	0.000	0.041	0.035	0.147	0.154	0.003	0.010	0.003	0.003	0.041	0.040	0.055	0.185
H-SCC	0.007	0.005	0.000	0.004	0.036	0.043	0.157	0.095	0.007	0.006	0.000	0.001	0.036	0.046	0.155	0.112	0.006	0.004	0.000	0.006	0.040	0.040	0.134	0.080
H-DSN %	0.005	0.006	0.004	0.000	0.038	0.041	0.113	0.136	0.007	0.007	0.001	0.000	0.034	0.040	0.170	0.139	0.005	0.001	0.004	0.004	0.040	0.052	0.098	0.024
H-NSS	0.008	0.005	0.000	0.002	0.039	0.041	0.171	0.103	0.006	0.007	0.000	0.001	0.036	0.042	0.147	0.139	0.010	0.003	0.002	0.004	0.042	0.039	0.180	0.072
F-ALTITUDE	0.006	0.006	0.003	0.000	0.040	0.039	0.119	0.139	0.006	0.006	0.002	0.000	0.040	0.038	0.126	0.134	0.005	0.003	0.004	0.003	0.040	0.044	0.102	0.056
F-LATITUDE	0.007	0.006	0.000	0.002	0.039	0.041	0.144	0.128	0.006	0.007	0.000	0.001	0.038	0.040	0.132	0.142	0.002	0.005	0.006	0.003	0.040	0.041	0.049	0.109

Especially for Mkg for all genetic line analyses (Table 17), heritabilities and additive-genetic variances were substantially larger in high productivity herds (group 2 for H-MPL). In addition, a higher percentage of DSN cows within herds (H-DSN % group 2) was associated with larger additive-genetic variances and heritabilities for Mkg. For Mixed, additive-genetic Mkg variances were 4.42 kg^2 in H-DSN % group 1, but 6.63 kg^2 in group 2 (Table 17). Corresponding values were 3.86 kg^2 in group 1 and 6.37 kg^2 in group 2 from the separate DSN analysis, and 4.62 kg^2 in group 1 and even 11.56 kg^2 in group 2 from the separate HF analysis. With regard to herd stratifications according to H-NSS, and according to classical environmental (geographical) descriptors F-ALTITUDE and F-LATITUDE, additive-genetic variances and heritabilities for Mkg were quite constant in groups 1 and 2 for all genetic line analyses. In HF, heritabilities and additive-genetic variances for Mkg were substantially higher in H-SCC group 1 (low SCC), but these estimates were almost identical in groups 1 and 2 for Mixed and for DSN.

Permanent environmental variance components for Mkg mostly followed the pattern of additive-genetic variances in groups 1 and 2 (Table 17): herd groups indicating a superior environment or herd management (larger herds, lower calving age, lower herd level for SCC, higher herd production level) not only had higher additive-genetic variances for Mkg, but also greater permanent environmental variances. In herd groups indicating suboptimal herd management, permanent environmental variances were smaller in the DSN and Mixed populations compared to corresponding estimates in HF. Estimates for permanent environmental variances for Mkg in DSN and Mixed were zero in group 1 for H-SIZE, in group 2 for H-CA and in group 1 for H-MPL, but were slightly higher in HF with 0.12 kg^2, 0.36 kg^2 and 0.01 kg^2, respectively. Apart from H-SCC, also residual variances for Mkg were larger in herd groups indicating a superior herd management (Table 17).

For test-day SCS, all genetic lines showed a similar trend for heritabilities and additive-genetic variances regarding herd descriptors H-SIZE, H-CA, H-MPL, H-SCC, F-ALTITUDE and F-LATITUDE (Table 18). Again, heritabilities and additive-genetic variances were higher in herd groups indicating a superior herd management, i.e., in larger herds allocated to group 2, in herds with a lower calving age allocated to group 1, in herds with a higher milk yield production level allocated to group 2, and in herds with a lower somatic cell count level allocated to group 1. Additive-genetic variances and heritabilities

for SCS within superior herd groups were larger for HF compared to corresponding groups in the DSN or Mixed analyses. The highest additive-genetic variance for SCS was 0.51, and the highest heritability was 0.16, both in HF for H-SIZE group 2. However, there were only minor heritability differences when comparing the three genetic lines, due to larger residual variances in HF. In contrast to Mkg, residual variances for SCS from all genetic line analyses were smaller in the superior herd groups compared to the respective suboptimal herd groups for H-SIZE, H-CA, H-MPL and H-SCC. Residual SCS variances for all genetic lines were larger for the groups with a lower SCS heritability, apart from the genetic herd descriptor H-NSS and the geographical descriptors F-ALTITUDE and F-LATITUDE. When grouping herds according to H-DSN %, quite small SCS heritabilities were estimated for both groups in the HF population (0.06 in group 1 and 0.01 in group 2), but in Mixed and DSN groups 2, additive-genetic variances (Mixed: 0.34, DSN: 0.35) and heritabilities (Mixed: 0.11, DSN: 0.12) were substantially higher than in HF.

For SCS, there was no obvious association between group estimates for the permanent environmental and for the additive-genetic variance component. In some scenarios, highest permanent environmental variances were identified in groups with largest additive-genetic variances, but also opposite relationships were found.

For test-day FPR (Table 19), in all groups and for all herd descriptors, additive-genetic variances were almost identical, especially for Mixed and DSN in a quite narrow range from 0.005 to 0.007. Hence, minor alterations in heritabilities for FPR were due to variations in the residual variance component. Again, also heritabilities for FPR were higher in the superior herd management groups for H-SIZE (group 2), for H-CA (group 1), for H-MPL (group 2) and for H-SCC (group 1). This was the case for all genetic line analyses. Quite similar variance components and heritabilities for FPR across groups and genetic lines were identified for the geographical descriptors F-ALTITUDE and F-LATITUDE, especially when comparing within-group estimates from the Mixed and DSN analyses. Interestingly, only for the HF analysis, the FPR heritability was substantially lower (0.02) in herds with a greater percentage of DSN cows (H-DSN % group 2), compared to the heritability in group 1 for H-DSN % (0.10). Such heritability differences for FPR between H-DSN % groups 1 and 2 were not observed for Mixed and DSN. Similarly, herd grouping according to H-NSS was associated with greater

heritability differences between group 1 and 2 in HF (0.18 and 0.07, respectively) compared to within-group estimates in Mixed and DSN. In general, heritability alterations were most obvious in HF (0.02 – 0.22), but in a narrow range in the Mixed line (0.09 – 0.17) and in DSN (0.11 – 0.17). For FPR, quite small permanent environmental variance components in the range from 0.000 to 0.006 were estimated for all groups across genetic lines.

Genetic correlations between the same traits recorded in different herd groups and clusters

Genetic correlations in same traits between groups 1 and 2 ranged from 0.08 (DSN, herd descriptor H-NSS) to 1.00 (Table 20). From 72 bivariate runs, 29 genetic correlations were exactly 1.00. Most of the "1.00 genetic correlations" were estimated for Mkg (result from 14 bivariate runs), being the trait with the highest heritability. Generally, genetic correlations were greater for Mkg (range from 0.60 to 1.00), compared to the estimates from the low heritability functional traits SCS (range from 0.08 to 1.00) and FPR (range from 0.43 to 1.00). Regarding genetic correlations for Mkg, estimates lower than 0.80 were found for the geographical descriptor F-LATITUDE (Mixed= 0.78, DSN= 0.70), and for the herd descriptor H-SIZE (HF= 0.60). Somatic cell score was the trait exhibiting the smallest genetic correlations, especially in the DSN analyses, when stratifying herds according to H-DSN % (0.11) or according to H-NSS (0.08). Furthermore, the stratification according to H-SIZE and H-CA with genetic correlation estimates lower than 0.78 indicated GxE interactions for Mixed (SCS, FPR) and DSN (SCS, FPR).

Table 20: Genetic correlations (r_g) between the same trait for milk yield (Mkg), somatic cell score (SCS) and fat-to-protein ratio (FPR) from herd groups 1 and 2 of the defined herd descriptors, stratified by genetic lines (Mixed: DSN + HF, DSN: German black and white dual-purpose cattle, HF: Holstein Friesian) (standard errors of r_g for all calculations <0.71).

	Mixed			DSN			HF		
Herd descriptor	Mkg	SCS	FPR	Mkg	SCS	FPR	Mkg	SCS	FPR
H-SIZE	1.00	0.63	0.75	1.00	0.57	0.78	0.60	0.80	0.94
H-CA	1.00	0.78	0.72	1.00	0.57	0.69	0.98	0.88	0.97
H-MPL	1.00	1.00	1.00	1.00	1.00	1.00	0.95	1.00	1.00
H-SCC	1.00	0.97	0.79	1.00	1.00	0.83	1.00	0.79	0.90
H-DSN %	1.00	0.14	0.91	1.00	0.11	0.84	1.00	0.94	1.00
H-NSS	1.00	0.49	0.43	0.91	0.08	0.97	0.85	1.00	0.48
F-ALTITUDE	0.94	0.77	0.98	0.90	1.00	1.00	1.00	0.86	1.00
F-LATITUDE	0.78	0.75	0.82	0.70	1.00	0.82	0.83	1.00	1.00

For all genetic lines, genetic correlations were quite large, when grouping herds according to herd means for productivity (H-MPL) and udder health (H-SCC). Genetic correlations ranged from 0.95 to 1.00 for H-MPL, and from 0.79 to 1.00 for H-SCC. For the herd management indicators H-SIZE and H-CA, genetic correlations were in a moderate to high range from 0.57 to 1.00. Quite high and stable genetic correlations were identified when grouping herds according to the geographical descriptors F-ALTITUDE and F-LATITUDE. Holstein Friesian only displayed a few genetic correlations lower than 0.80 indicating GxE interactions for the low heritability trait SCS and for FPR, when stratifying the data according to H-NSS. A larger proportion of genetic correlations lower than 0.80 was identified for the Mixed genetic line and for DSN.

Standard errors (SE) were quite small for genetic correlations close to 1, and also small for extremely low genetic correlations. However, substantial SE were identified for intermediate genetic correlation estimates, e.g. SE= 0.43 for r_g= 0.49 (H-NSS, Mixed).

Regarding the different clusters (Table 21) for the Mixed breed analyses, genetic correlations lower than 0.80 for Mkg were identified between cluster 1 and cluster 2 (0.74) and between cluster 1 and cluster 3 (0.70). In agreement with the across group analyses, genetic correlations were smaller for low heritability SCS (0.68 between cluster 1 and cluster 2, or only 0.49 between cluster 2 and cluster 3) compared to moderate heritability Mkg. Interestingly, there were no GxE interactions for FPR when allocating herds to different clusters, because genetic correlations were larger than 0.80: Genetic correlations were 0.81 between clusters 1 and 2, 0.95 between clusters 1 and 3 and 0.96 between clusters 2 and 3.

Table 21: Genetic correlations (r_g) between the same trait for milk yield (Mkg), somatic cell score (SCS) and fat-to-protein ratio (FPR) between clusters from the Mixed (black and white dual-purpose cattle, Holstein Friesian) breed analyses (standard errors of r_g for all calculations <0.49).

Trait	Cluster1 & Cluster2	Cluster1 & Cluster3	Cluster2 & Cluster3
Mkg	0.74	0.70	1.00
SCS	0.68	0.97	0.49
FPR	0.81	0.95	0.96

Genetic relationships within and between herd groups and clusters

Average relationships within herd descriptor groups for Mixed ranged from 2.42 % to 4.52 %, from 2.48 % to 8.66 % for DSN, and from 3.30 % to 9.34 % for HF (Figure 25). Genetic relationships within groups were always higher for HF and DSN compared to the

corresponding Mixed analyses. Apart from HF, relationships within group 1 or 2 were always larger than between group results. For HF, the 'between group relationships' were larger, compared to results within groups for the herd stratification according to H-MPL and H-NSS. As expected, a group stratification according to the genetic descriptor H-NSS was associated with quite large genetic relationships within group 1 (the group with a greater percentage of artificial insemination daughters), because a large fraction of cows in group 1 had the same sire. Additionally, great within group relationships for group 1 and 2 were identified for herd stratification according to herd management indicators H-MPL and H-CA.

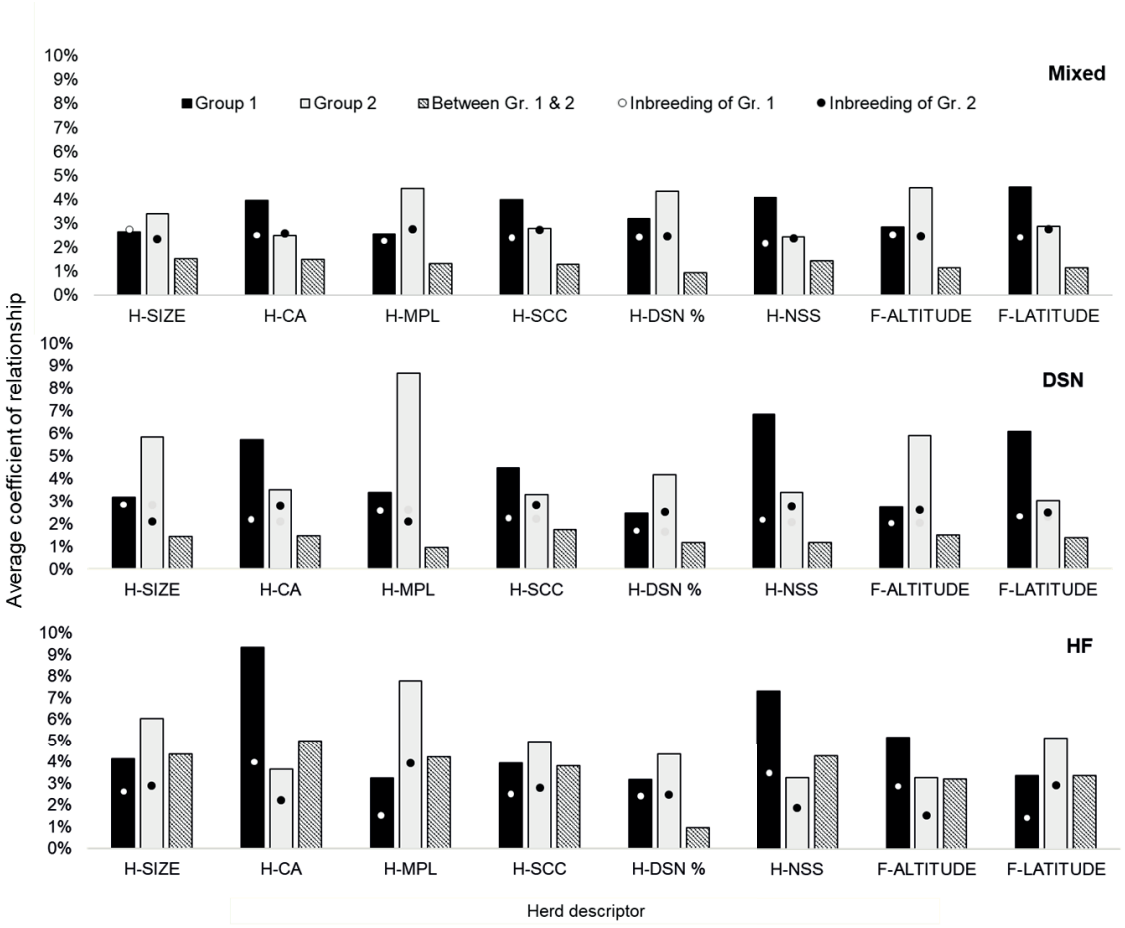

Figure 25: *Average coefficient of relationship (in %) and inbreeding coefficient (in %) of Mixed (DSN, HF), German black and white dual-purpose cattle (DSN) and Holstein Friesian (HF) between and within herd descriptor groups (as defined in Table 14).*

In most scenarios, stronger genetic relationships were found for Mixed, DSN and HF within groups representing the superior environment (H-MPL, group 2= high milk production level; H-SCC, group 1= low SCC; H-CA, group 1= low calving age; H-SIZE,

group 2= large herds). For Mixed, high genetic relationships were also observed for herds located 60 m above sea level (F-ALTITUDE, group 2= 4.48 %), and for herds located below a latitude of 52.2° (F-LATITUDE, group 1= 4.52 %). These two groups coincided with each other, as altitudes greater than 60 m above sea level mostly represented regions in the middle and the South of Germany, reflecting latitudes <52.2°.

Average relationships within clusters depicted a similar trend for all genetic lines (not shown). Highest coefficients of relationships within clusters were found for cluster 3 (Mixed= 8.70 %; DSN= 8.85 %; HF= 29.87 %), which only represented one large-scale farm from East Germany. As identified for the single herd descriptors, genetic relationships between clusters were lower than within clusters. The between-cluster relationships were largest between cluster 1 and 2 (Mixed= 2.60 %; DSN= 3.35 %; HF= 4.36 %).

Associations between genetic correlations and relationship coefficients

Figure 26 displays average coefficients of relationship between groups, based on a 'genetic correlation estimates' categorization. For same r_g-categories, coefficients of relationships were always largest for HF, but substantially lower and on a similar level for DSN and Mixed. Categorization of bivariate runs according to the r_g-categories 'r_g < 0.80', 'r_g 0.80 – 0.90' and 'r_g > 0.90' gave almost identical average coefficients of relationship between the groups for the same genetic lines. Hence, only a slight trend for larger additive-genetic relationships between groups with increasing r_g-estimates was identified, neglecting the hypothesis, that genetic connectedness between groups has an impact on r_g-estimates in MTAM.

Correlations between r_g-results with genetic relationships from the respective groups 1 and 2 were mostly close to zero. For the moderate heritability trait Mkg, correlations were not significantly different from zero (p> 0.05), and were 0.39 for Mixed, -0.02 for DSN, and -0.31 for HF. There was only a trend of stronger associations between r_g and group relationships for the low heritability trait SCS in the genetic line Mixed with a correlation coefficient of 0.55. For SCS, correlation coefficients were 0.37 in DSN, and 0.07 in HF. In addition, for the herd clustering approach, associations between r_g-results and "between-cluster relationships" were non-significant.

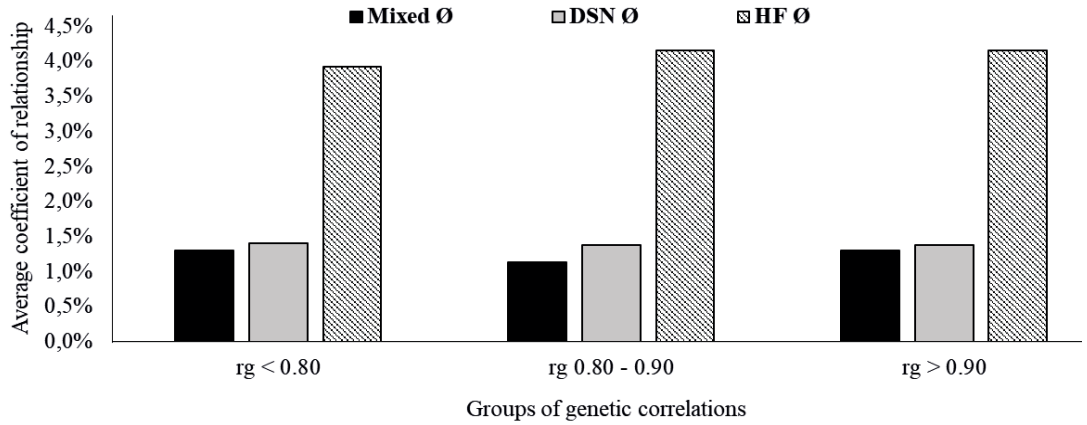

Figure 26: *Average coefficient of relationship (in %) between groups 1 and 2 (see Table 14) for different classifications of genetic correlation estimates within the genetic lines Mixed, DSN and HF.*

Discussion

Genetic variances and heritabilities within herd groups and clusters

In most cases, for all genetic lines and traits, heritabilities and additive-genetic variances were larger in herd groups reflecting superior herd management. In this study, 'superior management' included larger herd sizes and thus, automated farm processes, low calving ages, high milk yield and an improved udder health status. The results confirmed findings by König et al. (2008) in HF populations, who identified superior management and low calving ages in large-scale herds, contributing to greater heritabilities and a pronounced genetic differentiation. Homogenous variance components and heritabilities across groups and genetic lines for F-ALTITUDE and F-LATITUDE suggest, that these geographic descriptors were not considerably different environments, causing distinct genetic control of traits. Greater contrasts in altitude (e.g. mountain versus lowland pasture) or latitude (e.g. northern temperate climate versus southern tropics) with distinct climatic zones might contribute to heterogeneous variance component estimates. Ojango and Pollott (2002) confirmed this assumption, describing distinct differences in genetic variances for milk yield between Holstein cattle in the United Kingdom and Kenya. Results from the present study revealed, that a single environmental descriptor F-ALTITUDE or F-LATITUDE was insufficient to explain heritability alterations. Environmental impact on genetic parameters as identified by Ojango and Pollott (2002)

was due to the combined effect of climate and geography. Fikse et al. (2003) identified genetic parameter variations when considering at least three environmental descriptors (within-herd standard deviation, peak milk yield, herd size) out of a set of fifteen possible herd descriptors. In this study, the impact of environmental descriptors on variance components partly differed for Mkg and SCS. Results from structural equation models suggested to consider trait specific environmental descriptors, in order to describe heterogeneity of variances depending on the trait of interest (Fikse et al., 2003).

In the DSN herds, that mostly reflect low-input or grassland production systems, heritabilities for a production trait (Mkg) were greater than for a health (SCS) or metabolic stress indicator (FPR) across all genetic lines. Nauta et al. (2006) described a similar trend for Holstein cows in organic and pasture-based production systems. In the present study, additive-genetic variances and heritabilities increased with increasing intra-herd DSN percentages (group 2 for H-DSN %). Herds only keeping DSN, or herds with a large percentage of DSN cows, traditionally breed black and white cows in grassland systems (Jaeger et al., 2018). In contrast, most of the herds with a small percentage of DSN cows recently converted from indoor to grazing systems. Against this background, one explanation could be an improved adaptability of cows from pure DSN herds to grassland systems, contributing to the full phenotypic expression of their true genetic potential. These findings are in line with observations by Hoffmann and Merilä (1999), who stated, that trait heritability estimates depend on adaptive selection strategies, being specific for specific environments. In herds with a larger percentage of HF cows (group 1 for H-DSN %), intensive selection on production traits was a major breeding strategy for decades (König et al., 2007). Following the theoretical principles of animal breeding, intensive selection decreases levels of additive-genetic variances of quantitative traits. Falconer and Mackay (1996) referred to genetic architectures of quantitative traits and mentioned the reduction of additive-genetic variance being proportional to the increase of inbreeding coefficients. Koenig and Simianer (2006) described a rapid increase of inbreeding in commercial cow herds with a large proportion of HF cows, which was accelerated due to genomic selection in HF herds in recent years.

Quite constant additive-genetic variances and heritabilities within group 1 and 2 for H-NSS, F-ALTITUDE and F-LATITUDE imply, that these chosen environments or herd descriptors did not contrast enough, in order to initiate different genetic mechanisms for

Mkg with environmental alterations. Hammami et al. (2009) stated, that minor environmental differences mainly contribute to scaling effects, implying quite constant variance ratios in different environments. Holstein Friesian cows showed a reduced genetic variation for Mkg in harsh or "challenging" environments (i.e., high intra-herd SCC in group 2), but a considerable larger heritability and additive-genetic variance for Mkg in H-SCC of group 1. Such results indicate environmental sensitivity for HF.

Regarding SCS, larger heritabilities within superior herd groups were not only due to larger additive-genetic variances, but also due to the smaller residual components. For SCS, environmental sensitivity (i.e., alterations of genetic variances with environmental alterations) was more pronounced in HF, compared to DSN or Mixed. Calus et al. (2006) made similar observations for high yielding HF at the first test-day early in lactation. They concluded, that the lower resistance against major pathogens of high yielding cows, early in lactation, increased mastitis incidences, contributing to increased variance components for SCS. On the other hand, small and quite constant SCS variance components, early in lactation for DSN, might be due to better adaptive mechanisms. Such genetic line differences with regard to endoparasite infections, and further impact on genetic parameter estimates were identified by May et al. (2017).

Regarding FPR and all genetic lines, extremely small permanent environmental variances within all groups were identified, suggesting limited within-cow variation across lactations due to specific environmental effects. Consequently, Buttchereit et al. (2011) focused on genetic variance components within lactation for high yielding HF and identified heritability alterations within lactation, due to biological processes with different genetic background in different lactation stages. In the present study, major impact on variations of heritability estimates across herd groups was due to alterations of the residual component.

Genetic correlations between herd groups, clusters and GxE interactions

Robertson (1959) suggested, that a genetic correlation equal or lower than 0.80 indicated possible GxE interactions. Genetic correlations between the same trait in group 1 and 2 for the same environmental or herd descriptor were between 0.08 and 1.00. Genetic correlations from the clustering approach ranged between 0.49 and 1.00. Genetic correlations for Mkg mostly exceeded 0.80 for all genetic lines and herd descriptors,

except for H-SIZE and F-LATITUDE. Correspondingly, Zwald et al. (2003) reported genetic correlations above the '0.80-threshold' for moderate heritability production traits. Hammami et al. (2009) stated, that differences in genetic (co)variance components for production traits were mostly due to scaling effects. They deduced, that small environmental differences within European countries do not justify separate breeding schemes in different environments. When stratifying the data via cluster analysis, genetic correlations for Mkg in the Mixed line were 0.74 between the first and second cluster, even lower between the first and the third cluster (0.70) and highest between the first and third cluster (1.00). Hence, as suggested by Fikse et al. (2003), considering several herd parameters simultaneously contributed to production characteristics, that are more diverse.

For low heritability SCS in all three genetic lines, the impact of environmental changes on genetic covariances and genetic correlations was more pronounced than for Mkg. These results are in agreement with findings of Calus et al. (2006), who identified genetic correlations in a broad range from 0.43 to 0.89 for SCS. Similar observations were made for low to moderate heritability FPR, with genetic correlations lower than 0.80 for different combinations of genetic lines and herd descriptors. For FPR, and in contrast to Mkg, genetic correlations between clusters were larger, compared to genetic correlation estimates from single herd descriptor analyses, encouraging deeper analyses in this regard.

Best herd descriptors for environmental sensitivity (i.e., small genetic correlations) for all genetic lines and traits were H-SIZE, H-CA and H-NSS. Accordingly, Osorio-Avalos et al. (2015) stated, that herd management systems and herd size were most efficient criteria for herd clustering. Particularly small genetic correlations for SCS and FPR for H-SIZE, H-CA, H-DSN % and H-NSS stratifications underline this assumption. Intra-herd first calving age also had a major impact on genetic covariances of test-day traits from HF cows in large-scale contract herds (Gernand et al., 2007). Opposite results reported Zwald et al. (2003), because they estimated large genetic correlations, when grouping herds according to age at first calving.

Most of the genetic correlations lower than 0.80 where detected for Mixed. In most cases, herd descriptor x trait-combinations, causing low genetic correlations, differed between DSN and HF. Differences in genetic correlation estimates suggested, that different herd

descriptors have different importance in different genetic lines of black and white dairy cattle. Regarding genetic correlations, one specific genetic line and trait combination (HF x Mkg) was more affected by a herd management descriptor (H-SIZE), while another genetic line-trait combination (DSN x SCS) was stronger affected by genetic herd descriptors (H-DSN % and H-NSS). Zwald et al. (2003) considered both, herd size and the percentage of North American HF genes as important herd descriptors for HF cows. These findings coincide with the estimates from the present study, with small genetic correlations, especially for classifications according to H-SIZE and H-DSN %.

Impact of genetic relationships on genetic correlations

Average relationship coefficients for DSN within different geographical regions were quite large for the region of East Germany (8.81 %), and for small-scale family farms located in the South of Germany (6.71 %). Relationship coefficients were substantially lower for DSN herds from intensive grassland systems in the North of Germany (3.04 %), probably due to the less intensive use of artificial insemination in this region. Especially, high DSN relationships within the eastern part of Germany, but low relationships between East Germany and northern (0.73 %) or southern Germany (1.40 %) reflected the impact of 41 years of separate DSN breeding in the former German democratic republic.

Stronger genetic relationships within herd descriptor groups and clusters than between group relationships depict the impact of management or environmental classifications on genetic herd compositions (Yin and König, 2018). Quite high within-group relationships for H-MPL group 2 for DSN and HF are due to intensive selection on milk yield in both genetic lines, leading to decreased genetic variability and strong genetic connectedness. Correspondingly, Koenig and Simianer (2006) identified strong associations between relationship coefficients and breeding values of production traits.

Results from the present study rejected the hypothesis, that group relationships have an impact on genetic correlation estimates. This was also an assumption phrased by König et al. (2002), because genetic correlations in protein yield between two regions were larger when basing the calculations on proven bulls with a large number of daughters instead of young bulls. Recently, Yin and König (2018) used genomic marker data, and they found changes in genetic correlations when stratifying groups according to genomic

intra-herd inbreeding coefficients. Such contradictive findings suggest ongoing studies in this regard.

Conclusion

In general, larger heritabilities and additive-genetic variances for all genetic lines (DSN, HF and Mixed) were detected within "superior" herd environments with higher average milk production level (> 30 kg/day), lower average calving age (< 38 months), larger herd sizes (> 250 dairy cows) and low average somatic cell count (< 200,000 cells/ml). For all genetic lines, heritabilities and additive-genetic variances were larger for Mkg, compared to the functional health indicators SCS and FPR.

Differences in additive-genetic variances and heritabilities in groups 1 and 2 for the same herd descriptor were stronger for HF compared to Mixed or DSN. In all genetic lines, permanent environmental variances were small, especially for SCS and FPR. Alterations in FPR heritabilities were mainly due to changes of residual variance components in herd groups 1 and 2. For Mkg, genetic correlations mostly exceeded the critical threshold of 0.80. Quite low genetic correlations for SCS and FPR were identified, when grouping herds according to H-SIZE and H-CA. For the geographical descriptors F-ALTITUDE and F-LATITUDE most of the genetic correlations were larger than 0.80. Genetic relationships differed between groups and clusters, but were not associated with genetic correlation estimates.

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CHAPTER 6

Simulation and Economic Evaluation of Black and White Dual-Purpose Cattle Breeding Programs

Introduction

The robust German dual-purpose black and white cattle (DSN) have their origin in northern Germany and the Netherlands (Brade and Brade, 2013). Increasing selection and the import of American Holstein Friesian (HF) semen as well as artificial insemination (AI) during the 1960'ies resulted into an overall reduction of genetic diversity of the global DSN population. In 2016, the TGRDEU announced the breed to be endangered-maintained with a population number of 2,847 registered animals in Germany (TGRDEU). The DSN cattle are predestined for low input pasture-based milk production. These characteristics are reflected in their breeding goals, as they are expected to be of good health, robustness, good fertility and an adequate development potential with high forage and dry matter intake capacity. Due to these qualities, around 47 % of German DSN cattle are nowadays kept in organic pasture-based production systems (Biedermann, 2003). The organically managed herds usually implement natural service sires (NSS), as a decrease of AI is desired (Harder et al., 2004), whereas in conventional managed DSN herds (predominantly found in East Germany) AI is applied (Jaeger et al., 2018). The situation is very different in conventional HF dairy cattle breeding, where AI predominates. The federal statistical office reported, that in 2016 of all German dairy cows, only 4.1 % were kept in organic farms (Destatis, 2018). As opposed to a rather small dual-purpose population, that is managed organically to a significant extend, breeding programs and institutions of high yielding modern dairy breeds are organized on a large and international scale. König et al. (2009) simulated a conventional progeny testing breeding program for German Holstein cattle with 100 % AI, which consisted of a population size of 100,000 cows and a usage of 50 young bulls per year.

A breeding program can be simulated either via a stochastic approach or follow the deterministic methodology. An advantage of the straightforward stochastic approach is the possibility of imitating the actual breeding program more precisely by simulating individual animals. However, compared to deterministic simulations little insight is gained, while time and computer power requirements are high and thus, unsuited for comparisons between alternative breeding schemes (Haile et al., 2011). The deterministic approach is based on deterministic equations and population parameters, in order to predict genetic gain and inbreeding, rather than mimicking a breeding program on an individual animal level. Reduced computation time and detailed information about

genetic gain, inbreeding and economic efficiency characterize this approach as a convenient tool for comparing and evaluating complex breeding scenarios (Haile et al., 2011).

In this study, the software package ZPLAN+ (Täubert et al., 2011) was introduced to model genetic gain for different breeding scenarios, selection responses for single traits and the return on investment adjusted for costs of 3,000 cows. ZPLAN+ covers a wide range of functions (e.g. implementing genomic information, applying multiple stage selection, optimization of side effects) compared to alternative software packages (e.g. SelAction), that consider only single fragments of breeding programs (i.e. selection index) (Rutten et al., 2002). A graphic user interface, a platform-independent software and a database driven web-application contribute to its user-friendly handling (Täubert et al., 2011). However, it is of major importance to treat absolute output values of ZPLAN+ with caution, as all described input parameters are supposed to reflect the future, which is difficult to predict. With 20 years' worth of planning horizon, uncertainties and deviations will remain (Steininger, 2011). However, simulating alternating breeding scenarios with similar input parameters in ZPLAN+ will generate reliable results, which can function as a basis for comparison and decision-making.

As this study aims at evaluating breeding plans and their economic efficiency of a small dual-purpose dairy cattle breed, the breeding goal does not solely consider production, but functional and health traits as well. With regard to the difficulty of predicting future consumer trends and demands and the breeders' habit of thinking in generations, emphasizing functional and health traits in breeding goals, to reduce production costs, has proven to be a wise long-term decision (Willam et al., 2002). Harder et al. (2004) shared this opinion as well, stating, that emphasizing functional traits within a breeding goal is a reasonable alternative and may present a possibility to reduce costs. Since the 1960ies, Scandinavian countries demonstrated, that the recording and adoption of reproduction and health traits into HF selection schemes led to improved functional efficiency of cows along with an increase in production (Philipsson and Lindhé, 2003). Including functional and reproduction traits in turn, will affect the genetic gain as well as the economic outcome of breeding programs, due to low heritabilities and unfavourable negative correlations between functional, reproduction and production traits (Willam et al., 2002). Willam et al. (2002) predicted a change in the design of dual-purpose Simmental progeny

testing schemes in dairy cattle. They concluded, that the inclusion of low heritable functional traits in the total merit index required more daughter records per sire in terms of breeding value accuracy. However, they demonstrated, that emphasizing functional traits in the breeding goal led to reduced negative genetic gains and a higher monetary genetic gain.

For this reason, several schemes of conventional (based on AI), organic (based on NSS) and combined breeding of the DSN cattle population were compared to optimize annual monetary genetic gain and discounted return while minimizing discounted costs. Ceteris paribus, varying genetic correlations with regard to genotype by environment interactions (GxE) and changes in AI percentage in different runs were investigated. The objective of the study was to evaluate the genetic and the economic efficiency as well as implications of four different breeding strategies for a small dual-purpose cattle population including functional, fertility and production traits.

Materials and Methods

Genetic parameters

In order to run ZPLAN+ a matrix with genetic correlations, heritabilities, phenotypic standard deviations and phenotypic correlations is required. The depicted information in Table 22 was identified via an extensive literature research including production (milk yield: MY, average body weight: AvgBW), fertility (days open: DO) and functional traits (clinical mastitis: CM, longevity: L) (Frevert et al., 2014; Berry et al., 2003; Kern et al., 2015) (Shabalina et al., 2018). Lower heritabilities were found for functional, health and fertility traits (L, CM, DO). Often unfavourable correlations between production and fertility or health traits were recorded. Whenever information about correlations between traits was missing from literature, correlations were assumed to be zero. Economic values were calculated by using 1 divided by the respective genetic standard deviation, to meet the assumption of equal economic weight for every trait in the overall breeding goal.

Table 22: Phenotypic standard deviation (SD), heritabilities (diagonal), genetic (above diagonal) and phenotypic (below diagonal) correlations between traits. Economic values (EV) were calculated based on equal economic weights for the traits.

Trait	MY	DO	CM	AvgBW	L	EV
Milk yield (MY)	**0.34**	0.93	0.04	-0.01[1]	0.23[7]	0.60
Days open (DO)	0.12	**0.03**	-0.18	-	-0.44[6]	-0.10
Clinical mastitis (CM)	0.01	0.02	**0.10**	-	-0.53[5]	-1.66
Av. body weight (AvgBW)	0.14[1]	-	-	**0.60[1]**	-0.31[2]	0.02[1]
Longevity (L)	0.05[7]	-	0.08[4]	-0.04[2]	**0.09[7]**	0.01
SD phenotype	2.88	60.57	1.91	47[3]	487.49[4]	

(Frevert et al., 2014); [1]= (Berry et al., 2003); [2]= (Kern et al., 2015); [3]= (Søndergaard et al., 2002); [4]= Shablina et al., 2018; [5]= (Roxström and Strandberg, 2002); [6]= (Zavadilová and Zink, 2013); [7]= (Jenko et al., 2015); - = not available from literature

Breeding strategies

For DSN, a population size of 3,000 dairy cows was assumed. Depending on the breeding program (BP), the number of test bulls per year and average number of daughter records for breeding programs changed, while keeping the dairy population constant. With regard to different BPs, selection groups with different selection intensities were formed.

Conventional breeding scheme. The first scenario reflected a conventional progeny testing approach (C_BP), described by Täubert et al. (2011) with 100 % AI and a waiting bull system with planned mating and a three-step selection of male offspring. The average number of daughter records for AI sires was 50. The C_BP consisted of four selection groups (bull calves/ young bulls, test bulls, AI proven sires, AI elite sires) for the selection of breeding bulls. As the DSN breed has a rather small population size, only a small number of sires was selected for progeny testing. The proportion of test bull candidates, selected for progeny testing per year was 0.38. From test bulls, 33 % became AI proven sires, based on their daughter performance records and another 40 % of the AI proven sires were chosen for AI elite sires. Population parameters of the C_BP are depicted in Table 23.

Conventional breeding scheme with GxE interactions. The second breeding strategy evaluated GxE interactions (GxE_BP), applying the C_BP assuming daughters of AI proven and elite sires were kept in different production systems (e.g. organic vs. conventional). Genotype by environment interactions were accounted for by varying phenotypic and genetic correlations between the same traits (e.g. Milk yield 1 and Milk yield 2) from 0.1 to 1.0 stepwise by 0.1 in separate scenarios.

Organic breeding scheme. The third scenario described an organic breeding plan (O_BP) with the implementation of natural service sires (NSS) and two selection stages for male offspring with an average number of five daughter records per selected young bull. In total, three selection groups (bull calves/young bulls, NSS, elite NSS) for the selection of breeding bulls were formed. A proportion of 33 % NSS was selected from bull calves/young bulls of which 20 % became elite NSS, based on their progeny performance records (Table 23).

Combining conventional and organic breeding schemes. The fourth breeding strategy combined the C_BP and O_BP (Figure 27). During this approach, the pedigree matrix based on changing percentages of male (NSS, elite NSS, AI proven sires, AI elite sires) and female (NSS, elite NSS, test bulls, AI proven sires, AI elite sires) selection groups on the sire selection path. While the percentage of AI and NSS varied within the male (MSG_BP), the female (FSG_BP) and both (male and female) selection groups (MFSG_BP), all other parameters of the C_BP and O_BP were kept constant. During the simulation of the MSG_BP scenario the percentages of 90 % AI sires (all proven sires) and 10 % NSS were changed in steps by 10 % until the last scenario; implementing 10 % AI sires and 90 % NSS (Table 24). In the FSG_BP, the percentages of AI sires changed from 90 % (1/3 proven sires and 2/3 test bulls) and 10 % NSS to the last scenario of 90 % NSS and 10 % AI sires (Table 24). During the MFSG_BP scenario percentages of male and female selection groups were changed simultaneously, starting with 90 % proven sires and 10 % NSS for the male selection group. Accordingly, the female selection group stepwise increased the NSS percentage and decreased the test bulls and proven sires' percentage, starting with 90 % proven sires (1/3) and test bulls (2/3) and 10 % NSS (Table 24).

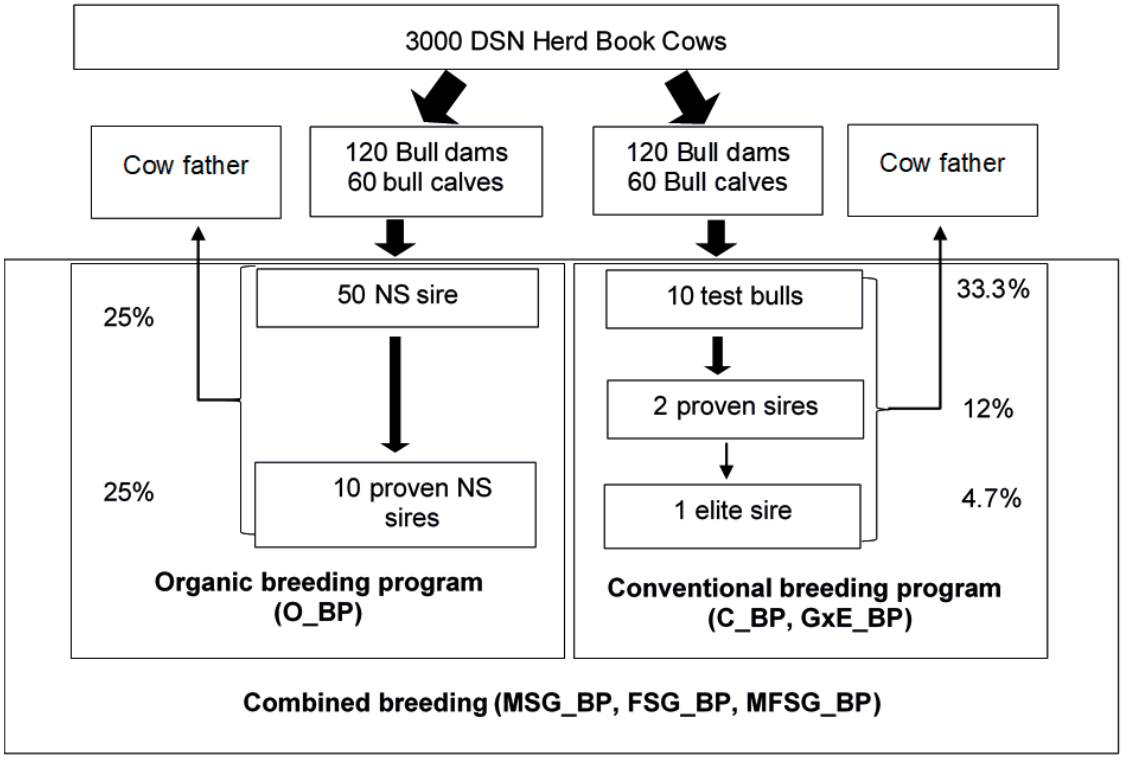

***Figure 27**: Combination of conventional (C_BP) and organic breeding (O_BP) programs (MSG_BP, FSG_BP, MFSG_BP), as well as the genotype by environment interaction breeding program (GxE_BP).*

Table 23: Population parameters of a conventional and an organic breeding program (BP) for the DSN cattle population applied in ZPLAN+.

Input parameters	Unit	Conventional BP	Organic BP
Total population size	number	3,060	3,200
Recorded cows	number	3,000	3,000
Proportion of AI	%	100	0
Proportion of test bulls	%	38	33
Test bulls/year	number	15	50
Bull dams/year	number	80	300
Old bulls/year	number	2	10
Bull sires/year	number	15	50
Calving interval	year	1	1
Productive life, young bulls	year	1	1
Productive life, test bulls	year	3	3
Productive life, proven/ elite bulls	year	3	3
Productive life, bull dams	year	4	4
Productive life, dams	year	4	4

Table 24: Different scenarios within the combined breeding program of conventional (C_BP) and organic (O_BP), simulating relations of male (MSG_BP), female (FSG_BP) and both (MFSG_BP) selection groups.

Breeding program	Selection group	NSS elite NSS	Test bulls	AI proven sires AI elite sires	cows
MSG_BP	Male change	10 %*	0 %	90 %*	100 %
	Female constant	50 %	30 %	20 %	100 %
FSG_BP	Male constant	50 %	0 %	50 %	100 %
	Female change	10 %*	60 %**	30 %***	100 %
MFSG_BP	Male change	10 %*	0 %	90 %*	100 %
	Female change	10 %*	60 %**	30 %***	100 %

*= relations changed from 10 % to 90 % and 90 % to 10 %. **= relations changed from 60 % to 7 %. ***=relations changed from 30 % to 3 %.

Breeding costs

In order to evaluate the economic efficiency of different breeding programs, relevant fix and variable costs need to be considered. Fix costs mainly included wages of breeding organizations and data processing. Depending on the population size and cows within one breeding stage, a decreasing trend in costs is desirable (Kalm and Harder, 2003). However, the described DSN population was rather small and due to this reason, fix costs were not considered in the calculations. Variable costs comprised expenses for performance testing, keeping test bulls, collecting/storing semen as well as body weight and milk recordings of cows. Average breeding cost components, that were accounted for in this study, based on Lind (2007), adjusted for current circumstances (small population) and inflation, are outlined in Table 25. The investment period covered 20 years with interest rates of 0.06 and of 0.04 for return and costs.

Table 25: Average variable breeding cost components for a conventional (C_BP) and an organic (O_BP) breeding program, adjusted for the present study based on Lind (2007).

Variable cost factors	C_BP (euro)	O_BP (euro)
Performance testing per cow	32	32
Weight recording of cows	18	18
Selection of bull calves	15	-
Test bull / nature service sire (total per animal)		
Price	2,000	2,000
Rearing, progeny-testing	13,000	-
Proven sire / proven nature service sire (3rd selection stage)	1,500	1,500

Evaluation of breeding strategies with ZPLAN+

The core of the program is based on a statistical-deterministic approach, a gene-flow matrix (Hill, 1974; Elsen and Mocquot, 1974; Brascamp, 1978) and the selection index theory (Hazel, 1943). The gene flow method allows to discount fluctuating breeding gain,

while analysing how genes are passed on from one selection stage to another. A selection stage includes all breeding animals of the same sex, productive lifetime, survival rate, generation interval, selection index and intensity. The analysis is based on the classification of the population into selection groups in order to set up the gene flow matrix (Kalm and Harder, 2003). The user defines the breeding program to be analysed and all remaining necessary parameters. The program then calculates different evaluation criteria, such as the annual genetic gains for breeding scenarios, discounted returns and discounted costs for a certain investment period.

The annual monetary genetic gain (AMGG) was used to compare the four DSN breeding strategies. The selection responses for production and functional traits were analysed including the total discounted return (DR) and total discounted costs (DC). Selection responses in all figures were depicted in genetic standard deviations instead of absolute values of selection responses, to facilitate comparisons between scenarios. The AMGG expressed the monetary superiority per year of the progeny of the selected animals. The DR described the monetary value of genetic superiority, reflected by improved animals within the population over 20 years (Willam et al., 2002).

Results

Comparison of conventional and organic breeding plans

The generation interval differed distinctly between the C_BP (4.83 years) and O_BP (2.75 years). Although the generation interval was almost halved when applying the O_BP. The undiscounted AMGG of the breeding goal did not vary considerably from each other. The C_BP (0.73) reached a slightly higher AMGG, than the O_BP (0.72) with greater response to selection for DO and L (Figure 28). The DR was marginally higher for the O_BP with greater response to selection per genetic standard deviation for MY and AvgBW, while the selection response of CM negligibly differed between the two scenarios (Figure 28). The variable DC per animal were 109.96 euro and 49.53 euro for the C_BP and O_BP respectively.

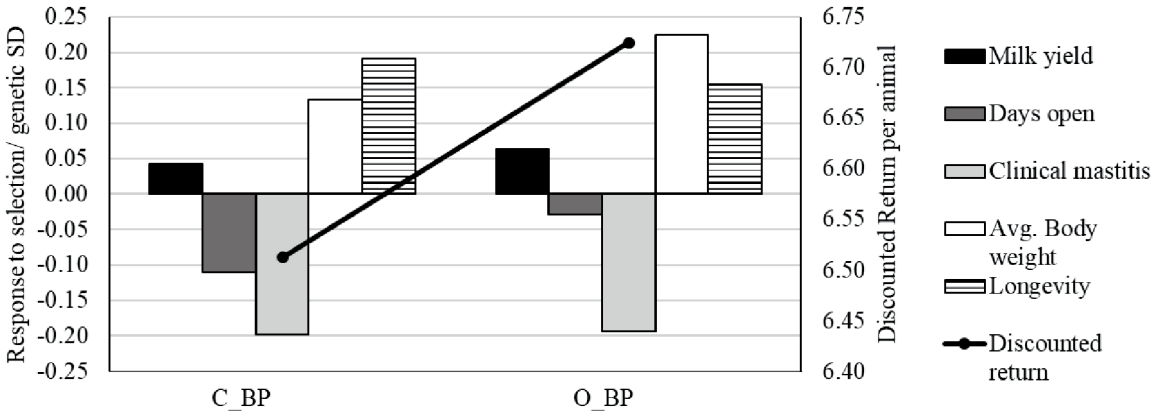

Figure 28: *Responses to selection of milk yield, days open, clinical mastitis, avg. body weight and longevity in terms of genetic standard deviations and total discounted return per animal (when equal economic weight was assumed) for the conventional (C_BP) and the organic (O_BP) breeding program.*

Comparison of conventional breeding plan with genotype by environment interaction

When simulating the influence of GxE interactions in the conventional progeny testing breeding program, stepwise decreasing genetic and phenotypic correlations by 0.01, the generation interval did not change (4.83 years). With decreasing correlations within the same trait, the response to selection showed decreasing trends of DO, AvgBW, and L, while MY increased and CM remained more or less stable. At a genetic correlation of 0.50, the selection response of AvgBW and L became negative. The highest DR per animal was realized in C_BP (no GxE interaction) and lowest DR was observed at a genetic correlation of 0.30 (Figure 29). The DC stayed at 109.96 euro per cow for all genetic correlations.

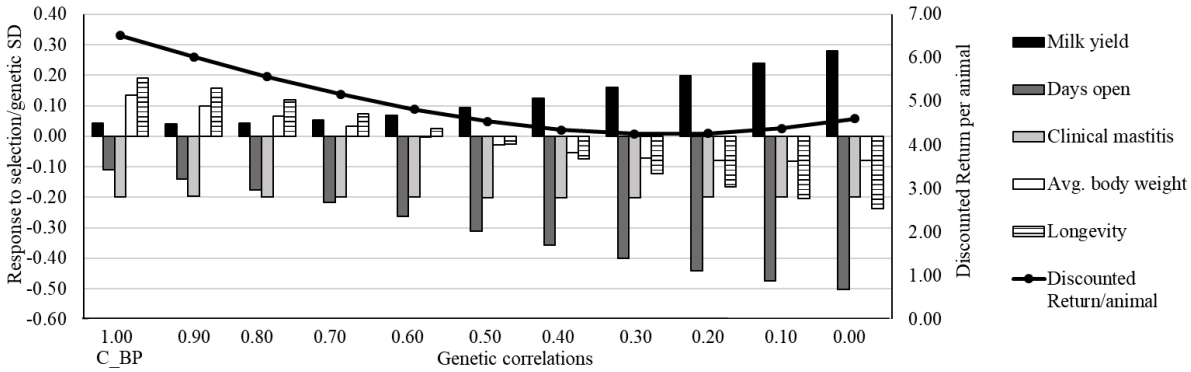

Figure 29: GxE_BP: *Responses to selection in terms of genetic standard deviations and total discounted return per animal (when equal economic weight was assumed) across different correlations within the traits milk yield, days open, clinical mastitis, average body weight and longevity.*

Comparison of combining the conventional and organic breeding plans

Male. Changing the selection stages of breeding sires in the male selection group (MSG_BP), the generation interval decreased with increasing percentage of NSS implementation from 4.23 down to 3.17 years. There were no changes in selection responses for MY throughout the different scenarios (0.08 kg). Days open remained negative but increased from -1.13 days to -0.86 days. Clinical mastitis and L decreased insignificantly, while the AvgBW increased from 5.36 kg to 7.07 kg. The maximum DR per animal unit was reached when 90 % breeding sires came from NSS and only 10 % from AI sires (Figure 30). The DR differed considerably between changing AI sire and NSS implementation combinations. The DC stayed the same throughout the simulations.

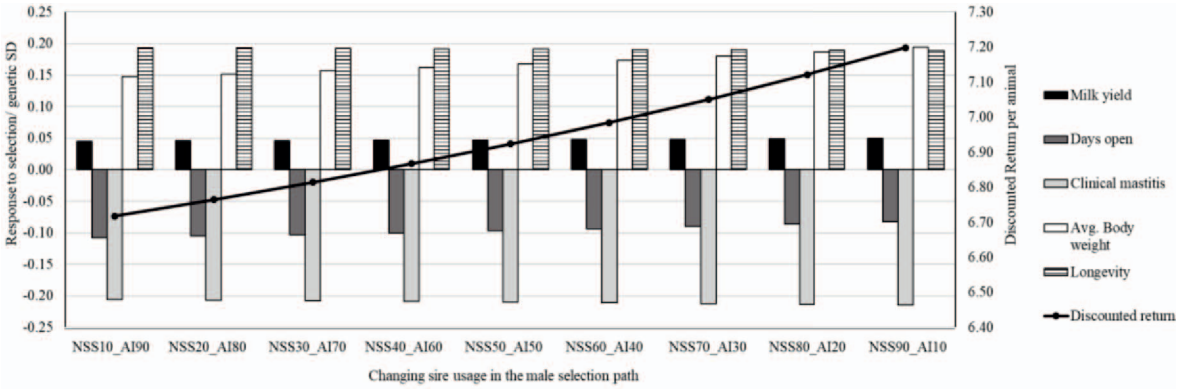

Figure 30: MSG_BP: *Response to selection in terms of genetic standard deviations and total discounted return per animal (when equal economic weight was assumed) across changing percentages of AI and NS sire implementation for the traits milk yield, days open, clinical mastitis, avg. body weight and longevity (→ keeping female constant, changing male).*

Female. In the female selection group (FSG_BP) the generation interval decreased slightly from 3.96 to 3.46 years with increasing utilization of breeding bulls from NSS. The selection responses of CM and AvgBW increased slightly, while L decreased from 29.65 days down to 26.09 days. In contrast to MSG_BP, the maximum DR for FSG_BP was reached for 10 % NSS and 90 % AI sires (60 % test bulls and 30 % proven sires) in the female selection path (Figure 31). The DC remained stable at 92.43 euro per animal.

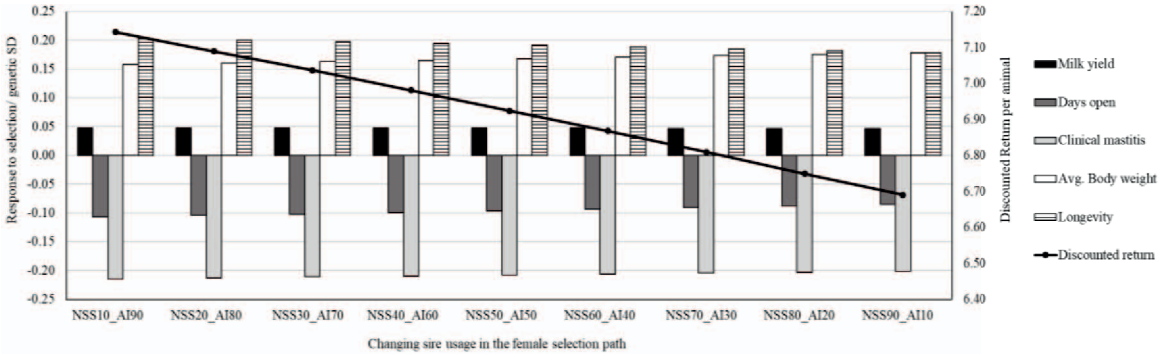

Figure 31: FSG_BP: *Responses to selection in terms of genetic standard deviations and total discounted return per animal (when equal economic weight was assumed) across changing percentages of AI and NS sire implementation for the traits milk yield, days open, clinical mastitis, avg. body weight and longevity (→ keeping male constant, changing female).*

Both. The MFSG_BP showed a decrease of the average generation interval from 4.5 years down to 2.9 years with increasing number of NSS in the breeding plan. The responses to selection were comparable to the outcome of the FSG_BP. The highest DR per animal unit (6.99 euro) was reached when 90 % of breeding bulls came from NSS and 10 % from AI sires (6.7 % test bull and 3.3 % proven sires) (Figure 32). However, a DR of similar range (6.95 euro per animal) was generated with breeding bulls of 10 % NSS and 90 % AI sires. The lowest DR was observed for the scenario with 50 % NSS, 33.3 % test bulls and 16.7 % AI proven sires. The DC per animal were the same as for the MSG_BP and FSG_BP scenario and did not change throughout the simulations.

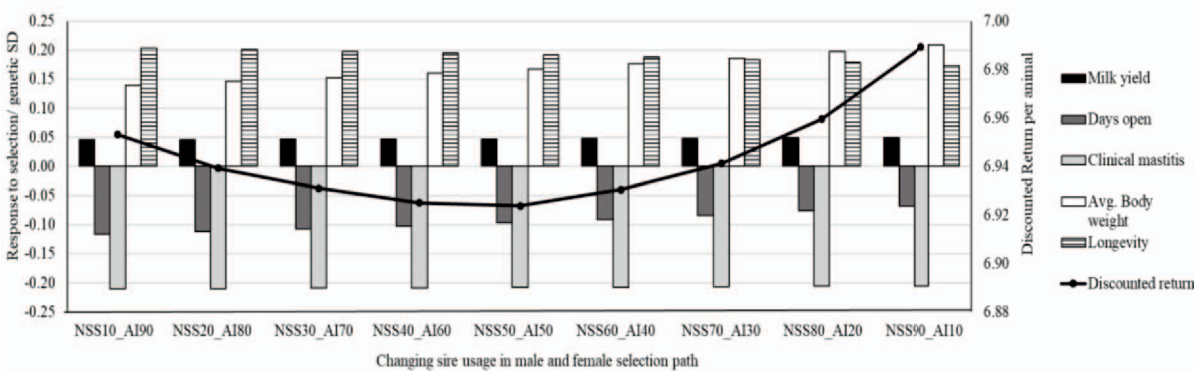

Figure 32: MFSG_BP: *Responses to selection in terms of genetic standard deviations and total discounted return per animal (when equal economic weight was assumed) across changing percentages of AI and NS sire implementation for the traits milk yield, days open, clinical mastitis, avg. body weight and longevity (→changing male and female).*

Comparing all breeding strategies, the greatest selection responses for MY and DO were found for the C_BP. For CM, the highest response to selection was found within the

MSG_BP (90 % NSS and 10 % AI sires) and within the FSG_BP (10 % NSS and 90 % AI sires). Average body weight showed the best selection response within the O_BP. Maximum values for selection responses of L (0.20) were observed in the MFSG_BP. The breeding strategies MSG_BP (90 % NSS and 10 % AI sires) and FSG_BP (10 % NSS and 90 % AI, and 20 % NSS and 80 % AI sires) reached the maximum AMGG of 0.79 (Table 26). The greatest DR per cow (7.20 euro) was observed for the MSG_BP (90 % NSS and 10 % AI sires), while the GxE_BP and the C_BP reflected the highest DC (109.96 euro).

Table 26: Annual monetary genetic gain (AMGG), discounted return (DR), discounted costs (DC) and discounted profits (DP) for different breeding simulations within programs. The conventional breeding program (C_BP) functions as the baseline scenario, which all other programs are compared against, with a generated discounted profit of= -103.45 euro.

Breeding plan	Scenario	AMGG, natural units	DR, euro	DC, euro	DP, euro
C_BP	none	0.73	6.51	109.96	0
O_BP	none	0.72	6.72	49.53	60.64
	r_g= 100 %	0.73	6.51	109.96	-0.00
	r_g= 90 %	0.68	6.02	109.96	-0.49
	r_g= 80 %	0.64	5.57	109.96	-0.94
GxE_BP	r_g= 70 %	0.61	5.16	109.96	-1.35
	r_g= 60 %	0.58	4.81	109.96	-1.70
	r_g= 50 %	0.56	4.54	109.96	-1.97
	r_g= 40 %	0.55	4.35	109.96	-2.17
	r_g= 30 %	0.56	4.25	109.96	-2.26
	r_g= 20 %	0.57	4.27	109.96	-2.25
	r_g= 10 %	0.6	4.38	109.96	-2.13
	r_g= 0 %	0.64	4.6	109.96	-1.91
	NSS10_AI90	0.76	6.72	92.43	17.73
	NSS20_AI80	0.76	6.76	92.43	17.78
	NSS30_AI70	0.76	6.81	92.43	17.83
	NSS40_AI60	0.77	6.87	92.43	17.88
MSG_BP	NSS50_AI50	0.77	6.92	92.43	17.94
	NSS60_AI40	0.77	6.98	92.43	18.00
	NSS70_AI30	0.78	7.05	92.43	18.07
	NSS80_AI20	0.78	7.12	92.43	18.14
	NSS90_AI10	0.79	7.2	92.43	18.21
	NSS10_AI90	0.79	7.14	92.43	18.16
	NSS20_AI80	0.79	7.09	92.43	18.10
	NSS30_AI70	0.78	7.04	92.43	18.05
	NSS40_AI60	0.78	6.98	92.43	18.00
FSG_BP	NSS50_AI50	0.77	6.92	92.43	17.94
	NSS60_AI40	0.76	6.87	92.43	17.88
	NSS70_AI30	0.76	6.81	92.43	17.82
	NSS80_AI20	0.75	6.75	92.43	17.76
	NSS90_AI10	0.75	6.69	92.43	17.71
	NSS10_AI90	0.78	6.95	92.43	17.97
	NSS20_AI80	0.77	6.94	92.43	17.95
	NSS30_AI70	0.77	6.93	92.43	17.95
	NSS40_AI60	0.77	6.93	92.43	17.94
MFSG_BP	NSS50_AI50	0.77	6.92	92.43	17.94
	NSS60_AI40	0.77	6.93	92.43	17.95
	NSS70_AI30	0.77	6.94	92.43	17.96
	NSS80_AI20	0.76	6.96	92.43	17.98
	NSS90_AI10	0.76	6.99	92.43	18.01

Discussion

In the light of the present results, the important criteria determining the economic value (AMGG, DR and DC) of the described breeding strategies were a low generation interval, genetic correlations between traits, strong selection responses of functional traits (not production traits!) such as CM, AvgBW as well as L and in this context, a high number of daughter records per sire. Additionally, for a breeding program to succeed, the prevention of GxE interaction was considered fundamental as well as the overall population size.

Effect of generation interval on genetic gain and economic factors

The lowest average generation interval of all scenarios, found in the O_BP, can be explained with the missing selection stage of sires on testing stations. Natural service sires are implemented at the age of 12 months and are usually replaced when they are on average 3 years old, whereas proven and elite AI sires are between 5 and 10 years before being replaced by their offspring. With an investment duration of 20 years, 7.30 generations were realized for the O_BP, but only a maximum of 6.31 generations were reached conducting alternative strategies (MSG_BP with 90 % NSS and 10 AI). In this context, the superiority of the O_BP opposed to conventional progeny testing (C_BP) must be mentioned. The O_BP realized 3.13 more generations for selection than the C_BP along with reduced DC and a similar AMGG. Paying special regards to DC in terms of breeding program selection is essential. There are distinct warnings in literature against a large increase of costs in the breeding program, as this can be a significant barrier to the adoption of beneficial technology in breeding (Kahi et al., 2003). Although greater selection intensity with more selection stages was obtained in the C_BP, the AMGG might have been compromised by longer generation intervals. In the case of the present study, reduced selection intensity in the O_BP seemed to be compensated by almost doubling the generations for selection due to short generation intervals. Consequently, decreasing generation intervals in the O_BP appear to have a comparable effect on genetic responses for MY, CM and L as the enhanced selection of sires, i.e., realizing genetic superiority of AI sires in the C_BP.

The decline of generation interval could also be observed in the MSG_BP, the FSG_BP and the MFSG_BP along with decreasing implementation of AI sires, while extending

the use of NSS. Naturally, a stronger utilization of breeding bulls from NSS and elite NSS in the male and female selection group led to a reduced generation interval for sires. Harder et al. (2004) corroborated these findings explaining, that a higher proportion of NSS led to a decrease in generation interval. The reduced generation interval in the MSG_BP with the use of 90 % NSS and only 10 % AI sires can be one explanation for the obtained superior AMGG, DR and the high selection responses in CM, AvgBW and L. Kahi and Hirooka (2005) also expected faster genetic gains for carcass traits when decreasing the generation interval. Accordingly, other findings from literature accounted decreasing generation intervals for a higher discounted profit, due to minimizing costs for housing and feeding of waiting bulls, as well (Thomasen et al., 2014; Karras et al., 2011). However, the decreasing generation intervals did not seem to affect the AMGG of the FSG_BP positively. In this particular case, the AMGG declined with decreasing generation interval. Possible reasons may be, that in the male selection path AI and NSS remained constant at 50 % and the generation interval only decreased roughly one year from 4.23 to 3.17 years. In contrast to the MSG_BP, the FSG_BP reached the maximum AMGG with an 80-90 % implementation of AI sires. With regard to AMGG and DR, the MSG_BP constitutes a possible breeding strategy for DSN cattle and high NSS implementation.

Effect of number of daughter records and genetic correlations on genetic gain and economic factors in breeding programs

The number of daughter records per sire as well as genetic correlations are potential causes for varying selection pressure on functional traits in the presented breeding strategies. Compared to the O_BP, greater selection responses for DO, CM and L were observed in the MSG_BP and MFSG_BP. In turn, the O_BP realized a slightly higher selection response than the MSG_BP, FSG_BP and FMSG_BP in the production trait MY. The moderate higher genetic responses to selection for DO and L with increased AI sire implementation in the MSG_BP and the MFSG_BP could be due to the fact, that low heritable functional traits exhibit improved reliabilities of EBV in large scale progeny testing schemes, as opposed to elite NSS with limited daughter records. For the same reason the AMGG seemed to be higher in the FSG_BP, when implementing 90 % AI sires on the female selection path, leading to decreased DO and CM while increasing L. A similar effect was observed in the C_BP compared to the O_BP, where greater

responses to selection were observed for DO, CM and L in the C_BP (Figure 28). Moreover, it was confirmed that, with greater daughter size, higher genetic gain was generated for functional traits, while only low genetic gain was achieved for milk production (Sorensen et al., 1999; Harder et al., 2004). Harder et al. (2004) reasoned, that the optimum number of daughter records per test bull depended on the objective to be optimized. Although, the increase of their (Harder et al., 2004) number of daughter records up to 100 had only little effect on the monetary genetic gain of all traits, the composition of the monetary genetic gain was changed favouring the functional traits. Hence, they recommended around 100 or even more daughters per test bull for an organic breeding program (Harder et al., 2004). Regarding this aspect, Kahi and Hirooka (2005) stated, that the optimal test capacity and selection intensity depended on the breeding scheme and thus, with the level of recording. Optimal test capacity was high with low levels of recording and in case when young bulls were selected, based on their own performance alone. While including additional information from relatives of young bulls during first-stage selection process markedly decreased the optimal test capacity (Kahi and Hirooka, 2005). Willam et al. (2002) proposed an optimum number of daughters per test bull of around 100, while a further increase of number of daughters per test bull would only have little impact on the AMGG.

Moreover, the FSG_BP reflected the importance of sire selection. The implementation of 90-80 % AI sires, which possessed information from relatives, generated a superior AMGG and DR compared to all alternative breeding strategies except for the MSG_BP. Only minor differences were obtained between the economic outcome of the MSG_BP (90 % NSS, 10 % AI sire) and the FSG_BP (10 % NSS and 90 % AI sires). The AMGG and DC remain the same, while the DR is 0.06 euro less for the FSG_BP. The MSG_BP favours the implementation of NSS on the male selection path over AI sires, while the FSG_BP succeeds with a predominately AI sire implementation on the female selection path. Causes for this may be the low heritability of functional traits (DO: 0.03 h^2, CM: 0.10 h^2, L: 0.09 h^2) as well as their difference in positive genetic gain in the two breeding plans. While both breeding plans (MSG_BP: 90 % NSS and 10 % AI; and FSG_BP: 90 % AI and 10 % NSS) show a slightly positive genetic gain in CM (here decrease of CM), the MSG_BP has a lower generation interval as well as higher genetic gain for AvgBW (90 % NSS and 10 % AI). However, on the other side the FSG_BP (90 % AI and 10 %

NSS) has a superior genetic gain in DO and L while decreasing AvgBW. Thus, both breeding plans have their advantages and disadvantages in terms of positive genetic gain of functional traits and generation interval. However, they depicted opposite reactions (increase or decrease) of genetic gain in functional traits, due to unfavourable genetic correlations between the functional traits itself (DO & CM -0.18; DO & L -0.44; CM & L -0.53; AvgBW & L -0.31). This explains why the MSG_BP (90 % NSS and 10 % AI) and FSG_BP (90 % AI and 10 % NSS) reach similar AMGG but slightly different DR, as the genetic gain in functional traits differs as well as the generation interval. Kahi and Hirooka (2005) concluded, that focusing on sire selection led to the highest genetic response, rather than concentrating on cow selection to breed dams. Thus, a high percentage of AI sire implementation (90 %) on the female selection path in the FSG_BP and MFSG_BP improved the genetic gain of functional traits more than the genetic gain of the production trait MY.

One reason for reduced selection response in MY in all breeding programs, except the GxE_BP (for genetic correlations ≥ 0.50), could be unfavourable genetic correlations between production and functional traits (MY & DO 0.93; MY & CM 0.04; MY & AvgBW -0.01). In this context, Baumung et al. (2001) offered an explanation arguing, that the calculated expected selection responses for single traits were distinctly affected by their correlations. They concluded, that the lower selection response of milk traits in their study was nearly compensated by higher response of functional traits (Baumung et al., 2001). Kahi and Hirooka (2005) corroborated this argument stating that, the inclusion of some traits in a breeding goal may cause undesirable correlated changes in other sets of traits.

The effect of GxE interaction on genetic gain and economic factors

The AMGG and DR seemed to be affected by the presence of GxE interaction, as apart from the GxE_BP, all breeding strategies generated an AMGG > 0.72 and a DR > 6.51 euro. With decreasing correlations within the same trait, the AMGG and DR declined. The highest DR was generated when there were no GxE interactions present, which accorded to the simple progeny testing scheme of the C_BP. Especially, the functional traits (AvgBW and L) showed negative genetic responses to selection with decreasing genetic correlations, which apparently influenced the AMGG quite distinctly. Fuerst-

Waltl et al. (2002) explained a negative AMGG for the fertility trait DO with its limited heritability. They stated slightly negative AMGG for functional traits, such as paternal calving ease (-0.039) as well (Fuerst-Waltl et al., 2002). Concomitantly, Harder et al. (2004) described the importance of functional traits (somatic cell score and longevity) and their contribution to a sufficient AMGG. Accordingly, Willam et al. (2002) confirmed the relevance of functional longevity (as the economically most important functional trait) in the total merit index of Brown Swiss. Thus, declining AMGG and DR along with decreasing genetic correlations may come from the failure of cows expressing their genetic potential in a suboptimal environment. Whenever GxE interactions exist, individuals, that may be genetic superior in one environment, show average or even reduced performances in a different production system, resulting into decreased economic efficiency (Charagu and Peterson, 1998). Interestingly, the presence of GxE interaction (r_g= 0.00) caused high responses to selection for MY and DO, as higher economic values were assigned for MY, DO and CM. Possibly, diluting effects to unfavourable genetic correlations were another reason for high responses to selection for MY and DO. As the heritability for CM is smaller than for MY, the selection response remains smaller as well. However, a positive genetic gain of those traits did contribute neither to a satisfying AMGG (0.64) nor to a desirable DR (4.60 euro).

The effect of population size on genetic gain and economic factors

Due to the small population size of DSN cattle, DC per cow remained quite high (49.53 euro to 109.96 euro). Willam et al. (2002) showed the impact of population size on DP, comparing the Brown Swiss against the much greater Simmental population, leading to a 14–25 % lower DP for Brown Swiss. As mentioned at the beginning, no fix costs were included. The considerable difference of DC per animal between the O_BP and the remaining strategies resulted from the variable cost factors of rearing and progeny testing of waiting bulls (13,000 euro per waiting bull). Under different circumstances, i.e., greater population size, the combined breeding scenarios including AI (MSG_BP and FSG_BP) might be the better option with regard to AMGG, DR and DC. Harder et al. (2004) described controversial results as well, reporting a distinct superiority of a conventional as opposed to an organic breeding program. This was mainly due to a larger population size within the conventional breeding program, caused by improved selection of bull sires. They even suggested developing an organic breeding program throughout

Germany, to get an adequate population size of at least 50,000 cows (Harder et al., 2004). However, in a small population like DSN, the O_BP may be the best choice due low DC as well as generation intervals and satisfying AMGG at the same time.

König et al. (2009) considered a population of 100,000 Holstein cows, applying a conventional progeny testing program for 50 young bulls with 100 daughter records per year. They concluded, that in large dairy populations, too, the reduction of generation intervals due to genomic selection breeding programs and abdication of conventional progeny testing led to tremendous cost diminution and increased genetic gain (König et al., 2009). Consequently, greater population size will increase the benefits of economies of scale, reducing costs of progeny waiting bulls.

At this point however, the important matter of inbreeding must be considered as well. Even in big populations, findings from other studies warned against increasing selection intensity and decreasing generation intervals, regardless their impact on inbreeding (König et al., 2009; Hayes et al., 2009). Karras et al. (2011) reported increasing inbreeding in the sire selection path in combination with genomic selection and decreased generation intervals. Especially, in a small population with low generation intervals, increased inbreeding presents a major obstacle, particularly in the sire selection path. Therefore, regarding inbreeding, keeping more sires and implementing limited selection (i.e., O_BP) should be a feasible alternative for the small DSN population.

Conclusion

The lowest DC was realized in the O_BP, which together with the low generation interval resulted into a satisfying DR. From an economical point of view, the O_BP presented a favourable breeding strategy for a small cattle population emphasizing on functional traits as body weight while minimizing costs. As the C_BP represented a certain scenario of the GxE_BP (with a genetic correlation= 1), the GxE_BP (and thus the C_BP) was the least suitable breeding plan for a small dual-purpose cattle population with regard to economic measures. The selection scenarios of an 80-90 % AI sire and 20-10 % NSS implementation in the FSG_BP and a 10 % AI sire and 90 % NSS implementation in the MSG_BP provided a great opportunity, to significantly improve functional traits such as CM and L, while generating most desirable economic results regarding DR. Essential parameters affecting the economic parameters of the breeding programs were a reduced

generation interval, antagonistic genetic correlations between traits, strong selection responses of functional traits and high number of daughter records per sire while the absence of GxE interactions was considerable.

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CHAPTER 7

General Discussion

The development of a new merit index, or just the inclusion of new traits into an already existing breeding program, requires an extensive knowledge of genetic correlations between all traits. Otherwise, economic losses may be the consequence, due to unfavourable correlations among production and fertility or health traits (Philipsson et al., 1994). Hence, the aim of this chapter is the estimation of genetic correlations between production and sensor traits, exploring the relations between economically important traits and animal behaviour on a quantitative genetic scale. Furthermore, as the requirements of the dairy industry are many-sided, especially with regard to climate change, previously generated results and conclusions will be discussed in the context of future demands of cattle breeding. In the second part of this chapter, a more general focus will address environmental challenges, societal demands, and practical implementations of dairy farming, that require a minimization of its environmental footprint. As an example, the whole production system needs to reduce its overall water consumption, while feeding needs to be reconsidered, utilizing fodder that mitigates methane production (Augustin et al., 2013). In this regard, recommendations for breeders and farmers are given, using the example of DSN, to address the more than ever pressing and actual topic of the future direction, sustainable (dual-purpose) cattle breeding should take. Including innovative technology, such as herd management tools and future marker-assisted selection, the issue of organic livestock production 'how to improve animal husbandry and livestock health status', can be tackled. Thus, the potential of welfare and health trait indicators, as a breeding tool for defining new breeding goals, will be addressed.

Especially, which opportunities in breeding of dual-purpose cattle can be of good use and how to proceed from here onwards, will determine the focus of discussion with respect to the previously addressed chapters: 2) Maintaining genetic diversity and mitigating inbreeding in dual-purpose populations, 3) Applying innovative phenotypic behaviour trait recording in dual-purpose cattle, 4) Associating significant genomic variants with candidate genes of bovine behaviour, 5) Analysing GxE interactions in DSN cattle breeding, 6) Including functional traits in the economic evaluation of breeding programs for small dual-purpose cattle populations.

Genetic Associations between Productivity and Cow Behaviour

Introduction

One of the greatest challenges in farm animal breeding is the ability of breeders, to distinguish between traits, that are only temporarily favoured by the market and consumers, and those traits, which will be of long-term interest. In this regard, Lush (1960) concluded, "one would like to select today in accordance with the economic values, which will prevail 10 to 20 years from now". Planning ahead is particularly important, as the genetic choices, breeders make today, will affect the profit in future generations (VanRaden, 2004). Via breeding, the genetic merit of animals in future generations is changed, so they will produce the desired products more efficiently (compared to present generation) under future economic, natural and social conditions (Groen et al., 1997). In 1971, the first economic index was formed in the US, to include traits, that affected dairy cattle profit, while the national index of Sweden already consisted of 12 traits (milk production, growth rate, female fertility, stillbirth, ease of milking, temperament, six conformation traits) in 1975 (Philipsson et al., 1994; Philipsson and Lindhé, 2003). During the 1970ies and 1980ies, the American predominant selection focus lay on milk, fat, protein as well as cheese yield (Norman and Dickinson, 1971). Philipsson et al. (1994) reported, that the only national breeding index, incorporating health traits before 1994, were found in Scandinavia. One explanation for the overall lack of health and functional traits in breeding programs was the problem of accurate recording and genetic evaluation for low heritable traits (VanRaden, 2004). Nowadays, breeding for functional traits, in order to reduce expenses, is nearly as relevant as increasing income. Due to this reason, the USDA's net merit index was changed in 2003, to include genetic evaluations for calving ease and cow fertility (Philipsson and Lindhé, 2003).

With the rapid development of PDF and new herd management technologies, to track cow activity (e.g. pedometer, electronic ear tag sensors, electronic collars/halters), it became feasible to record large data volume of individual cattle behaviour and estimate genetic parameters of these traits (chapter 3). Whether sensor traits will be included into the total merit index of DSN breeding programs remains to be seen. Nonetheless, in order to develop a total merit index for cattle breeding, it is essential to be aware of the genetic correlations between all traits, that are included.

There are various theories, that aim to explain the occurrence and biological reason of negative genetic correlations. Rendel (1936) reasoned, that the cause of genetic correlations was due to traits sharing resources for their development. Hence, a limitation in resources would cause a negative correlation between those traits. Consequently, selecting one trait affected the total amount of available resources as well as their distribution (Rendel, 1936). On this basis, Goddard and Beilharz (1977) developed the 'Resource Allocation Theory', where resources (e.g. feed intake, body tissue), used for one biological process (maintenance, reproduction, reaction to pathogens and stressors), cannot be allocated to another process. Farm animals in particular are confronted with the issue of limited resources. Due to artificial selection, the residual feed intake (feed intake that is not required for maintenance and reproduction) is disproportionally used to the maximum development of one trait, leaving the animal little resources to adequately respond to unexpected environmental stressors (Dunnington, 1990). Rauw et al. (1998) argued, that a growing imbalance between fitness and production traits will result in increasing negative correlations and challenge the homeostatic balance within an animal. Thus, genetic selection for production traits alters the physiological system, consuming endogenous resources, that are needed to strengthen the animal's disease resistance, reproduction and metabolism.

In order to construct multiple-trait selection indices heritabilities, phenotypic and genetic correlations are needed, to maximize accuracy (Hazel, 1943; VanRaden, 2004). The relative efficiency of selection depends on the number of traits selected, relative economic values of traits, heritabilities, phenotypic and genetic correlations between traits (Young, 1961). Accordingly, negative correlations among favourable traits will reduce the genetic progress (Lin, 1978). Moreover, as genetic and environmental correlations may differ in magnitude and sign, the apparent phenotypic correlation does not necessarily give any inference about the magnitude and sign of the genetic correlation (Rauw et al., 1998). VanRaden (2004) explained, how health and fitness traits received less attention in the past, because accurate genetic evaluations were not available for low heritable traits. As a consequence, one-sided selection for yield and type traits led to a decline in fertility traits, due to unfavourable correlations with yield traits. To name only a few impacts of genetic selection, that affected the homeostatic balance of animals, Wall et al. (2003) emphasized the unfavourable genetic response of BCS when selecting for milk yield.

They implied, that sires with higher EBV had daughters with lower body condition, due to greater negative energy balance. A reduced negative energy balance in turn caused reduced fertility and health (Wall et al., 2003). Moreover, Shanks et al. (1978) reported increased joint and leg injuries in daughters of sires, that were predicted to have high average milk yield, compared to a control group of cows, that were randomly bred. Axiomatically, the problem of undesirable side-effects, that come along with one-sided selection, can be observed in other species as well. Breeding for high body weight in turkeys correlated with higher mortality rates, negative immune performances, such as lower antibody responses, as well as heart and circulation problems (Rauw et al., 1998). In Swedish pigs (Landrace, Yorkshire), selected for high leanness and growth rate, greater leg weakness and osteochondrosis scores were observed, confirming unfavourable genetic correlations between constitution and growth rate as well as between constitution and lean percentage (Lundeheim, 1987).

The previous chapters already emphasized the economic importance of functional traits and their integration into total merit indices, in order to reduce production costs. Especially, against the background of long-term selection decisions, that affect the course of breeding for several generations, it its crucial, to be aware of all correlations among traits, whether they are favourable or not. Hence, the aim of this study is the estimation of genetic as well as phenotypic correlations between sensor and production traits. This is the first study, in which phenotypic and genetic correlations of electronically recorded behaviour traits of different dual-purpose cattle breeds, kept in extensive grazing systems in different countries, were estimated. The innovative approach intends to contribute to a better understanding, of how correlations will affect future dual-purpose cattle breeding. Anticipating the expression of production and functional traits in future generations, will help to prevent undesirable responses of selection.

Materials and Methods

Test-day production and sensor records were available for dual-purpose cattle from Germany (DE_DSN= black and white dual-purpose cattle), Poland (PL_BS= Brown Swiss, PL_HF= Holstein Friesian), and Switzerland (CH_OBS= dual-purpose original Brown Swiss, CH_Si= Simmental) (Table 27). The data set was the same as for the estimation of genetic parameters of sensor traits, described in chapter 4, except for the Slovenian records. Test-day records included milk yield (Mkg), fat percentage (Fat%),

protein percentage (Pro%) and the log-transformed somatic cell count (somatic cell score= SCS), while the daily mean of sensor traits on the test-day was considered. In total, 243 cows were included, while the pedigree consisted of 8,798 animals. Estimation of variance components was accomplished in bivariate models, including the pedigree-based and genomic relationship matrix together, using the software package DMU (Madsen and Jensen, 2018):

Model for sensor traits: $y_{ijklm} = BC_i + YS_j + AgisAge_k + PE_l + A_m + e_{ijklm}$

Model for production traits: $y_{ijklmn} = BC_i + Lact_j + Calvcl_k + PE_l + A_m + DIM_n + e_{ijklmn}$

Where,

y_{ijklm}	=	RUM, FEED, NA, ACT, HA, ET, I, IC
y_{ijklmn}	=	Mkg, Fat%, Pro%, SCS
BC_i	=	Fixed effect of breed-farm (DE_DSN, PL_BS, PL_HF, CH_OBS, CH_Si)
YS_j	=	Fixed effect of year-season (summer2016, autumn2016, winter2016, winter2017, spring2017, summer2017, autumn2017, winter2018, spring2018)
$Lact_j$	=	Lactation number (1-12)
$AgisAge_k$	=	Regression of the age of the cows
$Calvcl_k$	=	Calving season (summer2015, autumn2015, winter2015, winter2016, spring 2016, summer2016, autumn2016, winter2017, spring2017, summer2017, winter2018)
DIM_n	=	Regression of days-in-milk
PE_l	=	Permanent environmental effect of repeated observations
A_m	=	Random additive genetic effect
$e_{ijklm(n)}$	=	Random residual effect

Table 27: Observation and number of animals per breed included in estimation of genetic (co)variance components.

Breed	Observation	Number
DE_DSN	577	69
PL_BS	299	29
PL_HF	584	58
CH_OBS	309	44
CH_Si	324	43

Phenotypic correlations were not calculated based on the raw data, but based on the variances generated via the bivariate repeatability models in DMU. Applying the following formula, the phenotypic correlations were calculated:

$$V_{phen} = \frac{\left(cov(a1, a2) + cov(pe1, pe2) + cov(e1, e2)\right)}{\sqrt{\left(\left(V_{a1} + V_{pe1} + V_e\right) \times \left(V_{a2} + V_{pe1} + V_{e2}\right)\right)}}$$

V_{a1} and V_{a2} are the genetic variances for trait 1 and 2, cov(a1, a2) are the additive genetic covariances between trait 1 and trait 2, V_{pe1} and V_{pe2} are the permanent environmental variances, cov(pe1, pe2) are the permanent environmental covariances between trait 1 and 2, V_{e1} and V_{e2} are the rest variances and cov(e1, e2) are the rest covariances between trait 1 and trait 2. Applying the 'delta' method in R, including first-order Taylor approximation (Gold et al., 2018), the standard errors of the phenotypic correlations were calculated.

Results and Discussion

Sensor traits

Estimations of phenotypic and genetic correlations among sensor traits and between sensor and production traits are depicted in Tables 28-30. Most phenotypic correlations were similar to the ones from chapter 3, despite the differences between the two data sets (e.g. records per animal, number of dual-purpose cattle, breed, country) (Table 28). Phenotypic correlations, within the same range of chapter 3 and the present estimations, were found between the traits ET and FEED ($r_{p\text{-}chapter3}$= -0.17, r_p= -0.16), RUM and FEED ($r_{p\text{-}chapter3}$= -0.14, r_p=-0.13), RUM and HA ($r_{p\text{-}chapter3}$= -0.25, r_p= -0.28), RUM and NA ($r_{p\text{-}chapter3}$= -0.43, r_p= -0.33), FEED and ACT ($r_{p\text{-}chapter3}$= -0.36, r_p= -0.40), HA and NA ($r_{p\text{-}chapter3}$= -0.17, r_p= -0.22) and HA and ACT ($r_{p\text{-}chapter3}$= 0.32, r_p= 0.18). One reason for similar correlations in two different data sets may be, the sensor data's collinearity, as already pointed out in chapter 3. Once, the animal showed enhanced sensor behaviour of one trait, all other behaviour traits decreased, except from ACT and HA as well as RUM and FEED. Whenever ACT increased, HA inclined as did RUM and FEED. Due to the collinear data structure of the sensor data, discussions about correlations of behaviour traits are to be considered with caution, as the way of data measuring does not allow the recording of several activities, which may potentially be expressed at the same time

(described in chapter 3 and 4). This does not apply for the recording of ET, however, which was measured parallel to sensor behaviour.

Aside from the data's collinearity, it can also be assumed that, common physiological processes play a role, leading to the similarity of phenotypic correlations between the two data sets. Referring to this, Grant and Albright (2001) described, how dairy cows generally spend roughly 3 to 5 h/d feeding, ruminate 7 to 10 h/d and require another 10 h/d of lying and/or resting time. Fregonesi et al. (2007) formulated, that lying times in lactating dairy cattle followed a diurnal pattern, inverse to that of feeding behaviour. Regardless of farm, breed or country effect, negative correlations were depicted between RUM and FEED or between NA and RUM for the data set of chapter 3 (only German DSN cows), as well as for the dual-purpose breeds from Germany, Switzerland and Poland (Table 27). These findings indicated, that dual-purpose cow FEED, RUM and NA behaviour cannot be conducted simultaneously. Schirmann et al. (2012) depicted, that periods where Holstein dairy cows spent more time ruminating, were associated with lower feeding times and lower DMI (r_p= -0.71 and r_p= -0.72, respectively). Consequently, on a daily basis one might expect a positive relationship between rumination and feeding, because greater feed intakes may require more rumination time to process. However, direct negative correlations between FEED and RUM can be explained because cows cannot ruminate and eat at the same time. Additionally, the influence of management processes as milking, time of feed delivery and feed push-up should not be underestimated in affecting animal behaviour rhythms of when they feed, ruminate or rest (Schirmann et al., 2012).

Correlations (phenotypic and genetic) between sensor and I or IC did not always converge. This is not peculiar, as I and IC comprised of the sum of all sensor behaviour traits together. At this point I and IC shall not be of further interest.

Table 28: Genetic (above diagonal) and phenotypic (below diagonal) correlations within sensor traits with standard errors in brackets.

	RUM	FEED	ACT	HA	NA	ET	I	IC
RUM		0.31 (0.69)	-0.78 (0.64)	-0.18 (0.51)	-0.34 (0.58)	nc	nc	nc
FEED	-0.13 (0.02)		-0.67 (0.29)	-0.02 (0.19)	-0.85 (0.09)	-0.33 (0.28)	-0.37 (0.28)	nc
ACT	-0.50 (0.01)	-0.40 (0.01)		0.04 (0.30)	0.49 (0.37)	-0.05 (0.48)	0.61 (0.49)	nc
HA	-0.28 (0.02)	-0.08 (0.02)	0.18 (0.02)		-0.33 (0.20)	0.30 (0.30)	0.28 (0.28)	-0.06 (0.25)
NA	-0.33 (0.02)	-0.59 (0.01)	0.06 (0.02)	-0.22 (0.02)		0.29 (0.35)	0.39 (0.35)	nc
ET	nc	-0.16 (0.02)	0.07 (0.02)	0.11 (0.02)	-0.01 (0.02)		0.23 (0.43)	-0.11 (0.39)
I	nc	-0.14 (0.01)	0.06 (0.01)	0.05 (0.01)	-0.09 (0.01)	0.07 (0.01)		-0.87 (0.08)
IC	nc	nc	nc	0.02 (0.01)	nc	-0.05 (0.01)	-0.77 (0.00)	

nc= did not converge

The genetic correlations among sensor traits showed much higher standard errors, which can be explained with the rather small sample size of dual-purpose cows. Phenotypic and genetic correlations did not necessarily coincide with one another, but in most cases, they concurred well (Table 28). Exceptions, where phenotypic and genetic correlations dissented from each other (without consideration of I and IC) were RUM and FEED (r_g= 0.31; r_p= -0.13), NA and ACT (r_g= 0.49; r_p= 0.06), ET and ACT (r_g= -0.05; r_p= 0.07), as well as ET and NA (r_g= 0.29; r_p= -0.01). Cheverud (1988) concluded, that genetic and phenotypic correlations showed only broadly similar patterns. Dissimilarities between phenotypic and genetic correlations might be due to biological causes, environmental effects or due to imprecise genetic correlation estimates, because of small sample sizes or sampling errors (Cheverud, 1988).

The negative genetic correlations between RUM and ACT (-0.78), RUM and HA (-0.18), FEED and ACT (-0.67), and FEED and NA (-0.85) were all moderate to strong. This implied, that dual-purpose cows, which spent an increasing amount of time ruminating, would spend less time being active or sleeping and vice versa. A similar line of reasoning was already suggested in chapter 3, where mixed model evaluations revealed, that increasing daily percentages of sleeping in DSN cows reduced the time available for RUM and FEED. In this regard, relations to milk production level and behaviour profiles were considered more extensively, as physiological connections were indicated in several studies. Accordingly, Soriani et al. (2013) depicted positive relations between milk yield and rumination time.

While dual-purpose cows expressed enhanced ACT, they also seemed to be sleeping (NA) more (0.49). Once they were highly active (HA), they showed reduced sleeping periods (-0.33). These results imply, that normal daily ACT behaviour of dual-purpose cows also requires enhanced sleeping behaviour. However, during oestrus or parturition (when enhanced HA is usually recorded), cows express excessive walking, mounting and overall restlessness behaviour, while the usual resting habits decrease (Walker et al., 2008).

Regarding the genetic correlations between ET and the behaviour traits FEED, ACT, HA and NA, physiological relations can be suspected. With increasing ET, the FEED behaviour decreased. West (2003) explained the physiological processes behind high outside temperatures, increasing body temperature and declined feed intake by the example of dairy cows in the south eastern United States. Especially lactating dairy cows create large quantities of metabolic heat, which leads to increasing core body temperatures. In order to prevent heat accumulation through metabolic heat production, the feed intake decreases. As physical activity increases heat production by skeletal muscles and body tissues, a positive correlation between sleeping/resting (NA) and increasing ET (0.29) is understandable (West, 2003). In order to avoid more endogenous heat production, cows reduce their activity behaviour by lying down, in order to dissipate heat. Additionally, lying down on concrete floors enhances physical means of cooling mechanisms (conduction) as well (West, 2003).
A positive correlation between ET and HA (0.30) can be explained with the afore mentioned increase of body temperature during physical activity (West, 2003). Increasing restlessness and movement of cows is usually observed shortly before parturition or oestrus, leading to increased ET. Moreover, Piccioni et al. (2003) observed an elevation of 1.3 °C every 21 days on the day of oestrus in adult cows. Consequently, there may be several physiological reasons for a positive correlation between ET and HA. For once, the body temperature of cows during oestrus is already increased and second, they exhibit restlessness and increased movement, that contributes to further heat accumulation.

Sensor and production traits

The phenotypic correlations between sensor and production traits remained in a low range (≤ 0.14) with acceptable standard errors (Table 29). The highest phenotypic correlations were estimated between Mkg and the sensor traits FEED, HA and NA as well as between

Fat% and NA, and Fat% and ET. Small correlations between Mkg and RUM (0.07), and Mkg and FEED (0.14) comply with the trend of the least square means of the mixed model analyses from chapter 3. In chapter 3, DSN cows with higher daily FEED or RUM percentages depicted greater milk yields. The current multi-breed sensor data set (Table 27) derived a low positive relation between rumination, feed intake and milk yield output as well. Harrison et al. (1990) found a close correlation (r_p= 0.88) between milk production and DMI.

The trend of a negative relation between HA and Mkg (-0.11) can be explained by physiological processes, that occur during oestrus, the period when enhanced HA is mainly recorded. Lopez et al. (2004) related a decreased milk production to increasing oestradiol concentrations (r_p= -0.57; p< 0.001) during this time. Extensive restlessness (HA) may contribute to reduced Mkg, as usual diurnal behaviour, such as RUM was observed to decrease during increased HA (-0.28) as well, and milk production depends on rumination activity (Soriani et al., 2013). Decreasing Mkg was also observed when diurnal NA increased (-0.12). In this case, the same argumentation, as already discussed for DSN in chapter 3, can be invoked. Dual-purpose cows, that spend increased diurnal time with resting, would spend less time with feeding or rumination. Accordingly, Moallem et al. (2010) observed, that a depression of rumination time lead to a reduction of DMI followed by a decline in milk yield.

Increasing resting periods (NA) were related to positive Fat% (0.13). This outcome was not observed in the DSN analysis of chapter 3. Further research is required, to elucidate, whether a physiological relation between resting periods and Fat% exists in dual-purpose cattle. Phenotypic correlations between Fat% and FEED (0.00), RUM (nc) and NA (0.13) of this study, did not coincide with the previous findings from chapter 3. A low negative relation between Fat% and ET (-0.11) and between Pro% and ET (-0.09) was observed. Several studies investigated negative effects of heat stress on lactating cows and their milk composition. Knapp and Grummer (1990) concluded, that heat stress decreased DMI, milk yield and fat kg per day.

The remaining phenotypic correlations were rather low, or did not converge (nc), which is, why they are of no further interest. Phenotypic and genetic correlation almost never coincided with each other in magnitude. Strong environmental influences as well as a small sample sizes may be reasons for these dissimilarities (Cheverud, 1988).

Table 29: Phenotypic correlations between sensor and production traits with standard errors in brackets.

	Mkg	Fat%	Pro%	SCS
RUM	0.07 (0.04)	nc	nc	0.01 (0.04)
FEED	0.14 (0.04)	0.00 (0.03)	-0.02 (0.03)	-0.06 (0.03)
ACT	-0.06 (0.04)	0.01 (0.03)	0.02 (0.04)	0.04 (0.04)
HA	-0.11 (0.04)	-0.03 (0.03)	0.04 (0.04)	-0.02 (0.04)
NA	-0.12 (0.04)	0.13 (0.03)	0.04 (0.04)	0.03 (0.04)
ET	0.07 (0.03)	-0.11 (0.03)	-0.09 (0.03)	-0.04 (0.03)
I	0.02 (0.03)	0.00 (0.03)	0.03 (0.03)	0.00 (0.03)
IC	-0.02 (0.03)	-0.02 (0.03)	-0.04 (0.03)	-0.01 (0.03)

nc= did not converge

The estimation of genetic correlations between sensor and production traits, of different dual-purpose breeds, ranged from low to high values (≥ -0.75 to ≤ 0.65) (Table 30). The standard errors were quite high, which was assumed to be related to the small sample size and lack of pedigree completeness. Although some genetic correlations appear to be physiological reasonable, such as the positive relation between RUM and Mkg (0.41), some results appear questionable. A low negative correlation between Mkg and FEED (-0.10) is difficult to understand. The results of the least square means of the linear mixed models on phenotypic sensor traits of chapter 3, depicted a different trend. Dual-purpose DSN cows, that expressed greater diurnal feeding times, had higher milk yields. Moreover, estimated genetic correlations between feed intake and milk yield (0.46 – 0.65) from other studies, conducted on dairy cattle, strongly disagreed with the current correlation (Veerkamp, 1998). Another uncertain result is the strong relation between ET and Mkg (0.61), or ET and SCS (-0.75). In both cases, the standard errors are extremely high. However, due to the small sample size and pedigree incompleteness, the range of standard errors is not surprising.

The study has shown, that the estimation of genetic correlations was possible in most cases, nonetheless further research on a genetic level in this regard is advisable. Some behaviour traits of dairy cattle, such as rumination, have been related with dairy cattle health in the past (Radostits et al., 2007) and, more recently, changes in rumination have been used to investigate the responses of dairy cattle to acute stressors (Schirmann et al., 2012) and disease (DeVries et al., 2009). Furthermore, negative influences of acute stress, diseases and high stocking densities on rumination activity have been observed (Soriani et al., 2013). Therefore, behaviour traits seem to have great potential to be included in future breeding programs.

Table 30: Genetic correlations between sensor and production traits with standard errors in brackets.

	Mkg	Fat%	Pro%	SCS
RUM	0.41 (0.37)	nc	nc	0.53 (0.77)
FEED	-0.10 (0.31)	0.19 (0.37)	0.08 (0.29)	0.53 (0.66)
ACT	-0.11 (0.34)	-0.19 (0.46)	0.24 (0.32)	-0.60 (0.61)
HA	-0.32 (0.27)	-0.04 (0.37)	0.17 (0.27)	-0.71 (0.43)
NA	0.09 (0.33)	0.59 (0.49)	0.11 (0.34)	0.00 (0.61)
ET	0.61 (0.44)	-0.34 (0.59)	0.08 (0.42)	-0.75 (0.99)
I	0.24 (0.40)	-0.17 (0.51)	0.25 (0.43)	-0.01 (0.76)
IC	-0.30 (0.36)	0.14 (0.46)	-0.46 (0.36)	-0.65 (0.86)

nc= did not converge

Conclusion

It was shown, that phenotypic and genetic correlations of sensor and production traits often differed from each other. Hence, the phenotypic correlation did not necessarily give an indication about the magnitude of the genetic correlation between the same traits. Reasons for discrepancies are presumed to be due to environmental effects, biological causes, sampling errors, or inaccurate genetic correlations. While the standard errors of phenotypic correlations were acceptable, the standard errors of genetic correlations were, too high, in order to derive reliable conclusions. Next to the small sample size and pedigree incompleteness, another reason could be the colinear data structure of the electronically recorded traits. As the sensor system does not measure several behaviours, that are potentially expressed at the same time, but only records one, underlying genetic correlations may not be captured by this way of recording. Conducting further GWAS, as presented in chapter 4, on a more extensive data base might complement future examinations in this regard.

On a phenotypic level, the correlations of two different data sets coincided well. The smaller data set of chapter 3 consisted only of DSN cattle, while the multi-breed data set included additional dual-purpose breeds from other countries as well. Similar phenotypic correlations among sensor traits for both data sets were explained with biological causes (e.g. cows cannot feed and ruminate at the same time) and management routines (e.g. time of feeding, milking), that would affect similar behaviour expressions and thus, phenotypic correlations.

Phenotypic correlations between sensor and production traits depicted similar trends to the results of mixed model analyses, that were conducted in chapter 3. From this study, it can be concluded, that phenotypic correlations among sensor and between sensor and production traits showed encouraging results, while the estimation of genetic correlations, most likely requires a more extensive data base (greater sample size) as well as a more profound pedigree.

Future Perspectives

Maintaining genetic diversity and mitigating inbreeding in small dual-purpose cattle populations

Despite the rather low inbreeding coefficient of 2 % and average increase of inbreeding (0.1 % per year) for DSN, inbreeding depression is of minor importance. However, in a small population, with limited numbers of herd book cows and breeding sires, the rate of inbreeding should be monitored carefully. The Food and Agricultural Organization guidelines strictly recommend to avoid an inbreeding rate of >1 % per generation, to ensure fitness in a breed (FAO, 1998). Accordingly, Falconer (1989) emphasized the negative consequences of loss in genetic variability. He pointed out the increased risks of individuals inheriting detrimental or even lethal recessive genes, due to the homozygous state and furthermore, the effect of inbreeding depression, leading to decreases in performance of production, fertility and health. The effective DSN population size (85 animals) was lower, than for the German red and white dual-purpose cattle (Addo et al., 2017), which emphasized the urgency to monitor this breed. Although, the effective population sizes of Irish Hereford (64), Simmental (127), and Holstein Friesian (75) are within the same range as German DSN (Mc Parland et al., 2007), it needs to be considered, that DSN only consisted of about 2,722 herd book cows in 2013 (GEH, 2018). Moreover, the high average relationship (chapter 2) between breeding sires, with high genetic milk merit (Bedo, Nero, Best) and high yielding cows, implied a rather distinct breeding focus on production. Mc Parland et al. (2007), observed a similar phenomenon in Irish Holstein Friesian and Charolais cattle. Within those breeds, some ancestors accounted for a large proportion of the population, while the rest was related to many others (Mc Parland et al., 2007).

In the future, countermeasures should be taken, to attenuate this trend in DSN breeding, e.g. apply alternative breeding strategies, in order to maintain long-term genetic diversity. This could be realized by focusing on other breeding goals, aside from production, and the implementation of broader selection indices. Thus, alternative DSN family lines may be explored, which particularly exceed in desirable functional traits (fertility, health traits, sensor behaviour traits). Another way would be a more frequent exchange of sires (natural service sires or semen) between East and West German breeding organisations. As pointed out in chapter 2, the different DSN breeding

approaches between East and West Germany were one reason for the generally low relationship between eastern and western herds (0.73 %) (chapter 5).

Another possibility to restrict further increase in relationship and inbreeding, while increasing genetic gain, would be the use of optimum genetic selection (Koenig and Simianer, 2006). The underlying objective of the Optimum Genetic Contribution theory (OGC) is the maximization of genetic gain, while restraining the rate of inbreeding and coancestry among selection candidates to a predefined value (Wooliams and Meuwissen, 1993). Meuwissen (1997) reported genetic gains to be 21 % to 60 % greater than selecting via BLUP-EBV at equal rates of inbreeding. Especially in small populations, optimum genetic selection showed advantages over BLUP selection. However, that advantage decreases with increasing population size (Meuwissen and Sonesson, 1998). Meuwissen and Sonesson (1998) recommended a population of 1,700 nucleus animals, in order for optimal contribution selection to be useful in practice. This marks OGC a suitable tool, to apply for such a small population as DSN.

A more global approach, to increase genetic diversity and mitigate inbreeding, would be the inclusion of Dutch, Polish or British Friesian sires in DSN mating programs, as black and white cattle share a common genetic background (Brade and Brade, 2013; Mügge et al., 1999). The developed algorithm, to calculate DSN breed percentages (chapter 2), already accounted for Dutch ancestors in the DSN pedigree, highlighting some genetic influence from the Netherlands. Furthermore, there has been exchanges of Dutch and German breeding animals until the 19th century, expressed by similar phenotypic and genotypic traits. At one point in history, the Dutch cattle population was almost entirely repopulated by German black and white cattle mass imports from the Holstein region, due to cattle plague epidemics in the 18th century (Brade and Brade, 2013). In addition, the PCA (chapter 4) confirmed close genetic relations between HF breeds, emphasizing their common origin in northern Germany and the Netherlands (North Sea region, East Friesland) (Lush et al., 1936). Thus, old genetic ties between European black and white cattle lines still exist and can be detected on a genomic level as well as traced via pedigree analyses. These genetic relations of black and white dual-purpose cattle across European countries could be a potential basis for a transnational breeding program. Hinrichs et al. (2017) described, how the red dairy breeds are organized in transnational breeding programs. Such a European cooperation among black and white dual-purpose cattle breeders would allow a more frequent exchange of sires

and herd book cows between the Netherlands, Germany, Britain and Poland. This way, the future genetic diversity within the DSN population could be assured and the rate of inbreeding restricted, while still maintaining breed specific phenotypic characteristics.

Applying innovative phenotypic behaviour trait recording in dual-purpose cattle

The implementation of the sensor system has not only proven to deliver reliable behaviour data, but also allowed to derive management recommendations. This enabled herd managers to react to unusual animal behaviour, in case of distress, at an early stage. Hence, this system provides a valuable tool, to characterize dual-purpose cattle behaviour and to assess the animal's health and welfare state. Additionally, high data volume and repeated measurements per cow provided the basis for quantitative genetic estimations, also for a small number of cows with phenotypes. The results of chapter 3 were beneficial, as they support the system's functionality in dual-purpose cattle, kept in extensive grazing systems.

However, test runs of the system have shown, that updates were necessary to adjust the sensor technology to pasture conditions. Grodkowski et al. (2017) described rather poor correlations between visual observation and sensor recordings, on pasture during summer. They explained, that cattle expressed increasing head shaking and ear flapping, due to flies, which falsified sensor measures. When this was communicated to the company, an update of the system was developed, that, after installation, ensured high correlations between visual observation and electronic recording (e.g. rumination, r_p= 0.97, not active, r_p= 0.90) (Grodkowski et al., 2017). This points out, that environmental descriptors are not to be underestimated in studies, diverging from conventional frame of references. In other words, new herd management technology always needs to be validated, especially when applied in harsh environments outside the barn. After critical investigations, implementing a novel herd management tool in pasture systems has proven to be as helpful as in conventional systems.

Chapter 3 illustrated, how decreasing production was observed in dual-purpose cows, that were in distress, and depicted increased SCC levels due to (sub-) clinical mastitis. Hinrichs (2005) estimated a high genetic correlation (0.84) between SCS and mastitis susceptibility. This correlation points out the reliable detection of potentially sickened cows, with increased SCS/ SCC, by recording decreasing feeding and rumination times

via sensor. Especially in organic production systems, modern herd management technologies, that precisely monitor cow behaviour, are helpful in detecting diseases at an early stage. This is particularly essential, as organic farmers are limited in medication use (Vaarst and Bennedsgaard, 2001). Vaarst and Bennedsgaard (2001) explained, that the reduced need for disease treatment reflects animal health and welfare and that restrictions on medication in organic farming aim to encourage disease prevention. In Denmark, the shorter duration of antibiotic mastitis treatment in organic herds relates to restrictions and prohibitions to use follow up treatments with antibiotics (Vaarst and Bennedsgaard, 2001).

Learning more about the relation between genetic production potential and bovine behaviour will improve the understanding, of how dual-purpose cows adapt to a more extensive environment, cope with external stressors and how their behaviour affects the production output. Moreover, comparisons between the phenotypic sensor behaviour of dual-purpose cattle, in extensive systems, and high yielding dairy cows, in conventional farming, might offer insights to physiological (metabolic) processes, that function differently in high merit production and dual-purpose breeds.

Associating significant genomic variants with candidate genes

Detecting candidate genes of complex behaviour traits would allow breeders, to precisely select individuals early in life for genetic variants, that express desirable phenotypic behaviour in cows. The multi-breed GWAS identified significant SNPs for the MEAN and DRP of the sensor traits RUM, FEED and NA. Especially FEED has shown to be an interesting indicator for the estimation of milk production level in DSN (chapter 3), as cows with higher daily feeding times depicted greater milk yields. Applying marker assisted selection and genomic estimation of breeding values in the future is particularly tempting for traits, that are complicated to assess or are only observable late in live, which often applies for functional traits (e.g. longevity, robustness) (Goddard et al., 2010). Sensor traits revealed moderate heritabilities and additive genetic variances, demonstrating sufficient genetic control, that underlined cattle behaviour (chapter 4). Therefore, selection of these novel functional traits, based on phenotypic records and pedigree, is possible. Although, the identification of causative SNPs and candidate genes has proven to be complicated, there are some promising findings in literature regarding

significant associations between SNPs and functional (body temperature, feed intake) as well as production (milk quality) traits.

Liu et al. (2011) identified cows, that were genetically superior in body temperature regulation by virtue of inheriting a specific *ATP1A1* allele. They observed, that Holstein cows with genotype CC at the novel SNP of *ATP1A1* expressed a significantly higher heat resistance, than those of genotype CA. Extreme examples about dairy cows adapted to heat stress support the existence of genomic variants, that underlie the expression of temperature regulating traits in cattle. In order to detect significant SNP marker, the multi-breed GWAS should be extended to other dairy cattle populations with increased genetic diversity and cattle known for their heat tolerance, which are kept in tropical environments. This way SNPs, that are associated with temperature regulating genes, might be discovered.

Xi et al. (2015) associated the *PRKCI* gene with the hormonal regulation of residual feed intake (RFI) in Holstein cattle. Evidence of this study suggested, that genes regulating the adipocytokine signalling pathways and insulin pathway (e.g. *PRKCI*) were differentially expressed in the low RFI and the high RFI cow group. Furthermore, they explained, how insulin played a major role in the glucose metabolism, energy storage in insulin-sensitive tissues (muscle, adipose tissue) and its effect on RFI. Xi et al. (2015) concluded, that RFI was associated with the insulin signalling pathway and related it to the regulation of leptin and lipid metabolism. Corresponding to these findings, it could be hypothesized, that dual-purpose cows with a favourable expression of the *PRKCI* gene might show a more favourable feeding behaviour (3-5h per day, chapters 3 and 4) as well as RFI.

Accordingly, Nkrumah et al. (2004) discovered a SNP in the bovine leptin gene (obese gene), that was associated with feed intake, feed efficiency, feeding behaviour and body composition in Angus, Hereford, Limousin, Gelbvieh and Charolais cattle. They observed, that Angus and Hereford carried higher frequencies of the thymine (mutant) allele, while the beef lines, descending from continental breeds, depicted higher frequencies of the cytosine (normal) allele. The thymine allele was significantly associated with higher backfat gain (measured via ultrasound), poorer yield grades and lower lean meat yields, compared to the cytosine allele. Additionally, a trend of greater feed intake and feed efficiency was expressed in animals with the thymine allele

(Nkrumah et al., 2004). Further research, based on the sensor trait FEED, seems particularly interesting for DSN, as a dual-purpose breed. Findings suggest, that potential mutations in the obese gene may also be found in DSN, as they were once bred for beef as well (Mügge et al., 1999). This hypothesis is further supported by the estimated genetic relationships, based on ASD (chapter 4) between DSN and Hereford as well as between DSN and Angus. Thus, DSN might also have inherited the mutant thymine allele.
A study by Do et al. (2013) has shown, that SNPs in pigs existed within regions, where QTLs have been identified to be involved in feeding behaviour and/ or intake traits. They reported, that synapse genes, dephosphorylation genes and positive regulation of peptide secretion genes were significantly associated with feeding behaviour traits in pigs (Do et al., 2013). Identifying significant SNPs in the swine genome, that are potentially related to feeding behaviour, gives cause to believe, that similar SNPs potentially exist in the bovine genome as well.

Even in the area of milk composition and quality, information about genes and physiological pathways, that harbour genetic mutations affecting milk volume and composition is limited (Raven et al., 2016). Once again, the difficulty lies in narrowing regions to single gene candidates, due to the low level but long-range LD within cattle breeds and small sample sizes (de Roos et al., 2008). Furthermore, Dickson et al. (2010) discussed the challenge in identifying rare variants at high significant thresholds, which are required to avoid high false discovery rates. Raven et al. (2016) detected associations between SNP or indel in or close to *BTRC*, *MGST1*, *SLC37A1*, *STAT5A*, *STAT5B*, *PAEP*, *VDR*, *CSF2RB*, *MUC1*, *NCF4*, *GHDC* and milk production traits, and *EPGN* for both milk production and calving interval in Holstein and Jersey cattle.
The study of Poulsen et al. (2013) identified genetic variants of caseins, that were associated with bovine milk coagulation in Danish Holstein, Danish Jersey and Swedish Red cows. They observed a positive effect of *CSN1S1 C*, *CSN2 B*, and *CSN3 B* on milk coagulation, whereas *CSN2 A*^{2} had a particularly negative effect. Although, superior milk coagulation was observed in Danish Jersey compared to Danish Holstein cows, further research in the coagulation potential of DSN is auspicious. Especially, against the background of organic milk production, the coagulation capacity of DSN should be further examined, as substantial variation among individual cows has been reported. Differences in coagulation quality influence cheese processing (e.g. curd firmness) and

often, if insufficient, requires the addition of unwanted additives. As milk coagulation is a trait influenced by additive genetic variation, Poulsen et al. (2013) recommended the monitoring of unfavourable variants, in order to prevent inadvertent selection of cows, producing milk of impaired coagulation characteristics.

Moreover, the protein variants of cows and their effect on human nutrition presents another interesting field of research. Most milk proteins are potential allergens, while the release of peptides with biological functions from milk proteins is assumed to affect human health (Caroli et al., 2009). Rando et al. (1998) identified the bovine *CSN1S1*G* to be associated with reduced expression of the specific allergenic protein. Bio active peptides (specific protein fragments) on the other hand, are suspected to negatively affect the nutritional value, such as increasing diabetes and heart disease (*CSN2*A1*, *CSN2*B* and *CSN2*C*) (Lorenzini et al., 2007; Caroli et al. 2009). Caroli et al. (2009) concluded, that milk produced from cows carrying certain genotypes are more suitable for human nutrition in specific pathology situations, than milk of other genotypes. Aside from the nutritional or cheese making value, milk protein varieties are also of high commercial interest. Thus, selecting for DSN with milk proteins of beneficial effects for the human diet presents a promising marketing option.

Goddard et al. (2010) suggested, that many SNPs have small effects on quantitative traits. Accordingly, the challenge lies within the correct identification of causal polymorphisms affecting complex traits. Furthermore, they argued, a small effective population size (N_e) implies, that the effective number of chromosome segments is small and the accuracy of estimating their effects is high. In other words, N_e affects the extent of linkage disequilibrium in a population (Wientjes et al., 2013). Consequently, cattle breeds with reduced N_e (~100) depict a large variation in relationship, that can be sufficiently estimated by 50,000 SNPs. Correspondingly, the genetic variance explained by the SNPs is close to the full genetic variance (VanRaden et al., 2009). Due to the small effective population size of DSN, further GWAS analyses, based on a greater sample size of genotypes and phenotypic sensor records, should be pursued. This way, the accuracy of chromosome segment effects can be increased and contribute to further refine causal variants of behaviour traits.

Another approach is to conduct a GWAS across dual-purpose breeds, which are closely related to one another, such as DSN, British, Dutch and Polish Friesian. Saatchi

et al. (2014) concluded, that the detection of false positive associations from GWAS mainly derived from stochastic noise. Additionally, patterns of correlation among loci and factors, responsible for trait variation, create indirect associations between markers and traits, where no causal relationship exists (due to population structure). Hence, they suggested to address the problem of false positives by increasing the sample size and thus, the statistical power. Solving the issue of false associations due to population structure can be achieved by validating identified QTL in an independent, but demographic similar population. Consequently, whenever the same marker is highly associated across different populations, it is likely to be in strong LD with the causal variant (Saatchi et al., 2014). Following this approach, Saatchi et al. (2014) discovered several important across-breed and breed-specific large-effect pleiotropic or closely linked QTL in Simmental, Angus, Hereford, Limousin, Red Angus, Gelbvieh, Brangus, Maine-Anjou, Shorthorn and Charolais (Saatchi et al., 2014). Including similar demographic breeds to DSN might contribute to the discovery of DSN-specific QTL as well.

The effect of GxE interaction in DSN cattle breeding

The results of chapter 5 have shown, that despite the indications for GxE interactions, which affected DSN dual-purpose cows in Germany, a re-ranking of breeding sires or a separate evaluation of breeding values, according to different environments, is not necessary. As Germany is a rather small country, the chosen environmental descriptors were not as much distinct from one another. According to Weigel and Rekaya (2000), herds from small, neighbouring countries may be much more similar with regard to management, climate, and genetic background, than herds far away from each other, but within the same large country (Weigel and Rekaya, 2000).

Nonetheless, the phenomenon of GxE interactions in dual-purpose cows in Germany should be considered more relevant than ever. Against the afore mentioned background of climate change, there are environmental stressors, that might affect strains of Holstein cows (DSN, HF) differently. The study of Al-Kanaan et al. (2016) described how, even in temperate zones of middle Europe, heat stress is a serious issue. The study revealed slightly stronger abnormal physiological reactions (increase in rectal temperature and skin surface temperature) in HF cows, than in dual-purpose DSN, towards increasing temperature humidity indices (THI). A higher average rectal temperature of HF was related to greater metabolic heat production of high yielding cattle

with larger body size (Al-Kanaan, 2016). Thus, GxE interactions for THI measurements in Germany could be identified and implied, that dual-purpose DSN cattle have a physiological advantage under heat stress, compared to high yielding HF.

Another potential GxE interaction, that affects cattle in pasture systems, is the disease pressure resulting from parasites. May et al. (2017) calculated heritabilities in different HF lines for gastrointestinal nematodes (0.05–0.06), liver fluke (0.33) and lungworm (0.05). They concluded, that the lowest infections were identified for New Zealand Holstein lines, that were specifically selected for pasture-based milk production (May et al., 2017). In this regard, low to moderate heritable resistance against parasites could be included in long-term DSN breeding goals, as it has proven successful in New Zealand HF.

Consequently, environmental stressors need to be considered in extensive farming systems, as they cause GxE interactions, affecting DSN dual-purpose cattle. Despite little GxE interactions in DSN on production traits, it was shown from literature and own research (chapter 5), that functional traits (rectal temperature, skin surface temperature, parasite resistance, SCS, fat-to-protein ratio) were affected by changing environmental descriptors, while some Holstein lines (New Zealand HF, DSN) expressed better coping mechanism towards external stress than others.

Including functional traits in the economic evaluation of breeding programs for small dual-purpose cattle populations

Economic evaluations of breeding programs (chapter 6) have shown, that for a small native breed, the organic breeding plan, based on natural service sire implementation, was promising. The combined breeding scenarios of conventional and organic (MSG_BP/ MFSG_BP: 80-90 % natural service sire, 20-10 % artificial insemination) were feasible as well, although they involved higher DC.

Including functional traits, such as fertility, robustness, fitness, parasite resistance, heat stress resistance, and behaviour, into selection schemes of dual-purpose cattle, kept in pasture systems, is indispensable. It is widely recognized, that despite low heritabilities, selecting health traits has proven to be beneficial in farm management and helps to reduce production costs (Philipsson and Lindhé, 2003). Nordic countries have demonstrated, how the incorporation of fertility and health traits in the total merit index, improved

maintaining functional efficiency of cows and simultaneously increased production (Philipsson and Lindhé, 2003).

Aside a further adoption of the 'Scandinavian' philosophy in dual-purpose cattle breeding, another category of characteristics should be considered. Often, in organic milk production, a "healthier" fatty acid profile of milk from pasture fed cows is promoted. Several studies have pointed out increased contents of the beneficial *cis*-9, *trans*-11 conjugated linoleic acids (CLA) in milk from cows, that are extensively fed on pasture (Auldist et al., 2002; Kay et al., 2005). These milk fat contents have not only shown anticarcinogenic effects in animal models, but are considered to have other health benefits as well (Corl et al., 2003). A plethora of data demonstrates actions of CLA to reduce atherosclerosis, onset of diabetes, and body fat mass (Belury, 2002). These positive milk characteristics, that come with pasture-based feeding, are potential market niches, which can be used, to market milk products from DSN in grazing systems.

A more breeding related, rather than management related, marketing opportunity for DSN breeders would be the selection of dual-purpose cows, which express the BB variant of the β-lactoglobulin (LG) and κ-casein (CN) phenotype. Corresponding to literature, milk from cows of the BB variant of β-LG is superior for the manufacture of cheese (Aleandri et al., 1990; Schaar et al., 1985). Accordingly, greater concentrations of protein and casein in milk from cows of the BB variant of κ-CN, compared with milk from cows of the AA variant of the κ-CN phenotype, was observed (McLean et al., 1984; Aleandri et al., 1990).

Including functional traits in dual-purpose breeding does not only improve the cows' fitness, but also offers new possibilities, to market dairy products of dual-purpose cattle, from extensive systems, distinctly from conventional systems. Different incentives were proposed, which could help DSN farmers to take advantages of market niches via management (feeding, pasture access) or breeding (functional traits, milk composition traits). Also, sensor traits (rumination, feeding, active, sleeping, high active, ear temperature) could be included in the breeding goal as desirable fitness characteristics. Chapter 4 has proven sufficient genetic variance and heritability of sensor traits, allowing selection. However, the sensor system may be too expensive in acquisition, discouraging farmers from implementing it. This makes it difficult, to take behaviour traits into account in breeding programs, as long as on farm-based longitudinal standardized recording is lacking.

Conclusion

The results of this study have shown, that there is great potential for dual-purpose DSN breeding with regard to the adoption of new breeding goals and selection for novel functional traits. The breed is well organized by its breeding organizations RBB and VEF-DSN. The simulations of breeding plans have shown, that under the given economic and political framework, DSN breeding has the capability to react and adapt to new trends and challenges in the dairy sector by successfully increasing genetic gain in production as well as functional traits. As DSN are considered robust and well adapted to extensive pasture systems and organic dairy farming, they have great potential to satisfy consumer demands for increasing animal welfare standards and organic milk products. This may benefit the overall image of dairy farming as well. Hence, in the future the implementation of a standardized estimation of breeding values for innovative traits, such as behaviour, is conceivable. The results of the multi-breed GWAS lay the basis for more profound investigations, regarding the genetic expression underlining bovine behaviour, which in the future might offer the opportunity to understand, anticipate and prevent negative side effects of selection.

The major results from this study are:

- Pronounced differences regarding animal allocations to breeds, based on either breed percentage or on officially assigned breed codes, were detected. A small number of influential sires depicted large genetic relationships to high-yielding DSN cows. The average inbreeding coefficient of DSN was quite low (2 %) in recent birth years. No inbreeding depression for DSN was observed.
- Phenotypic relations between bovine behaviour traits, recorded via sensors, exist and can be used to derive management decisions and have potential as an early warning system.
- On the basis of sensor records from multiple dual-purpose cattle breeds across country borders, genomic regions of interest and potential candidate genes for behaviour and welfare traits were identified. Sophisticated studies on genetic diversity in dual-purpose cattle breeds revealed a clear separation of those breeds. Genetic similarities were identified and explained in the context of the breed history.
- Genetic parameter estimates of sensor traits revealed low to moderate additive genetic variance and heritabilities.

- Multiple-trait herd cluster models have been developed, in order to study GxE interactions for DSN, HF and Mixed (HF+DSN) cattle across distinct production systems and environments (herd size, calving age, latitude of farm, milk production level, somatic cell count level, genetic DSN percentage, percentage of artificial insemination and percentage of natural service sires). For some functional traits (somatic cell score, fat to protein ratio) genetic correlations were lower than 0.80, indicating GxE.
- The simulation and economic evaluation of breeding programs for small dual-purpose cattle revealed satisfying genetic gains, discounted returns and the lowest discounted cost for an organic breeding plan.

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Formal Declaration

Erklärung gemäß der Promotionsordnung des Fachbereichs 09 vom 07. Juli 2004 § 17 (2)

„Ich erkläre: Ich habe die vorgelegte Dissertation selbständig und ohne unerlaubte fremde Hilfe und nur mit den Hilfen angefertigt, die ich in der Dissertation angegeben habe. Alle Textstellen, die wörtlich oder sinngemäß aus veröffentlichten Schriften entnommen sind, und alle Angaben, die auf mündlichen Auskünften beruhen, sind als solche kenntlich gemacht.

Bei den von mir durchgeführten und in der Dissertation erwähnten Untersuchungen habe ich die Grundsätze guter wissenschaftlicher Praxis, wie sie in der „Satzung der Justus-Liebig-Universität Gießen zur Sicherung guter wissenschaftlicher Praxis“ niedergelegt sind, eingehalten.“

Gießen, den 05. Oktober 2018

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Maria Helene Palesa Jaeger

Lebenslauf

Der Inhalt wurde aus Datenschutzgründen entfernt.

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